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Scientific exchanges must serve science, not the Soviet government

THE exile of Dr Andrei Sakharov, coming so soon after the Soviet invasion of Afghanistan, raises once again the problem of a scientist's duty to take a stance on world affairs. Ironically it comes this time in defence of a man whose actions, over the past twelve years, have made it inevitable that scientists should be expected, by the public at large, to have such a stance. Just earlier this week, he issued a statement from internal exile in Gor'kii, via his wife, calling for a fair and democratic society and the defence of human rights. At the same time he accused the Soviet government of repression.

It would be simplistic, and far from correct, to attribute to Sakharov's influence the current concern of scientists with the social consequences of their research. Nevertheless, the appearance in *samizdat* in 1968, and subsequent publication in the West, of Sakharov's "Thoughts on Progress, Coexistence and Intellectual Freedom" have added a new dimension to the public image of the research scientist. Instead of what Isaac Asimov had called the "Frankenstein syndrome", the public were presented with a nuclear physicist, "father of the Russian H-bomb" (a title which Sakharov himself has always denied) who had turned over all his earnings from the weapons programme to cancer research, and begun a lone campaign for increased intellectual freedom and international exchange as a means of overcoming international tensions and confrontation.

In later years, Sakharov was to criticise this essay as immature. Certainly, as he became more closely identified with the growing wave of intellectual unrest in the Soviet Union, his approach to the problems of *détente* became more profound and, perhaps, less trusting of official statements of intent. The appearance of his essay in 1968, the "year of Czechoslovakia", however, threw a fundamentally new light on the Soviet scientific establishment. Hitherto, dissent had largely been confined to literary and student milieux, and Soviet scientists had been seen, at any rate from abroad, as enjoying an elitist position, with all the many privileges that the "classless" Soviet society gives to those it wishes to cherish.

For a Soviet scientist to risk his job, position and freedom, for the sake of an ideal has led, inevitably, to a new focus on the problem of the scientist's personal responsibility for the use to which his or her research is put — and, more generally, his or her obligations to fellow scientists and to humanity at large.

Dismissal from employment compatible with Sakharov's background and abilities came swiftly, providing the Soviet media with the chance of presenting him as a "drop-out" who has done no real science for the past 12 years. Even if this were true — and the circumstances of his arrest last week, on the way to a physics seminar, suggest otherwise — Sakharov's former scientific achievements and his continuing membership of the Academy surely constitute at least as much a claim for support from the scientific community as they would in the case of an aged and long retired professor emeritus. The storm of protest telegrams from scientific bodies and individual scientists indicate that this obligation has been felt and answered.

What form, apart from verbal protests, should this concern over Sakharov take? Already the US government has cut back on

technology transfer to the Soviet Union — and this at a time when the new Five Year Plans are being completed — plans which would have originally assumed the availability of US knowhow and/or hardware. Although the Soviet government will admit only Olympic visitors this summer, there are a number of international conferences scheduled to take place in the Soviet Union, before and after the Olympic shut-down, as well as an impressive programme of exchange visits and joint projects. Most significant, next month, the Hamburg Scientific Forum meets to discuss the implementation, as regards science, of the Helsinki Accords.

Since the first plans were made for Hamburg, the irony of the case of Yuri Orlov, has been observed on a number of occasions. Orlov, a physicist and founder of the Moscow "Helsinki Monitoring Group" is in a Soviet labour camp precisely for attempting to report on breaches of these accords. A number of western human rights activists, notably Valentin Turchin, advocate boycott of Hamburg until such time that Orlov is free to attend. Sakharov's exile, they claim, makes the case for boycott even stronger. At the other extreme lies the "soft diplomacy" approach: keep the contacts going, and try to do what one can behind the scenes. A doctrine which can all too easily become an apologia for inaction.

It would be ironic if, in defence of Sakharov, the world scientific community denies itself vis-a-vis the Soviet Union, all forms of international exchange which Sakharov himself advocated. On the other hand, it would be idle to pretend that the reprisals against him make no difference.

If scientific exchanges are to continue, then they must be seen to serve science and scientists — not the propaganda interests of the Soviet government. This, surely, is the time to implement a policy of "accept no substitutes"; to turn away from conferences those uninvited participants who turn up, armed with an irrelevant and insignificant paper, in place of the invited speaker whose passport somehow failed to materialise; and to protest against the terms of the form, signed by every Soviet scientist leaving the country, which limits his discussions to materials already published or passed for publication in the "open journals", thus cutting out the discussion of work in progress which is one of the most valuable features of any scientific meeting. And, when attending conferences in the Soviet Union, to make every effort to contact one's opposite numbers who may have been excluded from the conference — even if taking place in their own speciality and in their own home town — as happened a few years ago to the Goldshtein brothers.

Finally, when the occasion arises in the course of such conferences and contacts, scientists should relentlessly press for the implementation of those human rights agreements for which Sakharov has become, *par excellence*, the symbol of the concerned scientist. Indeed, perhaps the greatest tribute to his work is that the Soviet authorities now expect scientists who go abroad for conferences or exchanges to be challenged on such matters. In signing the pledge form referred to above, the Soviet scientist officially commits himself to answering all such enquiries according to the principles laid down in the official Party press. □



United States

1981 budget reverses shift from physical sciences to life sciences

Defence research increases set the pace for a 13% growth in the R&D budget which President Carter is asking from Congress in 1981. **David Dickson** reports from Washington

THE Carter administration is seeking to use the 1981 budget to reverse what it sees as a "disturbing trend" in science policy over the past decade and a half, namely a relative decline in funding for research in mathematics and the physical sciences compared to the growth in medical research and life sciences.

Official calculators, for example, show that support for physics dropped by 13.5% in real terms between 1967 and 1980, and for mathematics by 16.7% over this period. In contrast funding for the life sciences increased by 19%, and the environmental sciences experienced a real growth of 33.7%.

Moves to correct this imbalance — for example within the budget of the National Science Foundation, which plans to give particular emphasis to increasing support for fields such as computer science — figure prominently in a budget request also

characterised by a growth in research projects jointly sponsored by the federal government and private industry, and dominated by significant increases in Defense Department expenditure on research.

Overall President Carter, who singled out basic research in his budget message to Congress last Monday, as one of the few areas meriting increased support, is asking Congress for a 13% growth in support for research and development, including a 12% increase in funds for basic research.

Given an officially-estimated increase in cost of 9% (a figure some claim to be overly-optimistic) this will mean real growth in 1981 of 4% and 3% respectively in the total R&D and basic research expenditures.

Much of this is directly due to increases in the military research budget. Military sponsored R&D is planned to grow by

20.2% — including funds for research in very high speed integrated circuits and for full scale development of the MX intercontinental ballistic missile — and military support for basic research will be up by 21.4%.

The growth in the budget of the National Aeronautics and Space Administration will be comparatively smaller, at 9.8% for overall R&D (taking into account an extra \$300 million being requested for 1980), and 11.5% for space science.

However Dr Robert Frosch, Administrator of NASA, calls it a "good start" to the decade, with growth in all areas of the budget, at least compared to last year when the extra costs of the space shuttle meant no new space science programme starts.

Included in NASA's request for 1981 are funds for a gamma ray observatory, and an operational National Oceanic Satellite System (NOSS), which will build on the success of last year's SEASAT mission, and will be jointly funded with the Department of Defense and the National

Energy department announces 'slip' in laser fusion

THE Department of Energy has decided to allow "slippage" in construction work on the NOVA neodymium glass laser and irradiation facility at the University of California's Lawrence Livermore Laboratory in order to ensure a "more balanced" programme of advanced driver studies for inertial confinement fusion technology.

Construction work on NOVA started last year, and over \$80 million will have been spent on the project by the end of the current fiscal year. However in its budget request for 1981, the department has included no extra construction money — although it says that construction work will continue. Final costs are estimated at \$136 million.

In contrast to inertial confinement, the budget for magnetic fusion research has been increased by 13.5%, to a total of \$403.6 million. This figure includes a 40% increase for development and technology, and a 36% increase for applied plasma physics.

NSF to fund laboratory renovations

THE National Science Foundation is planning to introduce a new programme to help universities renovate out-dated

research facilities. Federal funding for the first year of the programme will be \$14.3 million, and the money will be awarded on a matching-grant basis, with academic institutions matching federal support with funds from non-federal sources.

According to NSF director Dr Richard Atkinson, the money will not be available to buy new instrumentation or equipment, or to restock libraries, but is intended to be spent on the improvement of existing facilities, on the basis that outdated facilities "bring less than optimal return on the investment of federal dollars for support of research". A statement from the Office of Science and Technology Policy says that the cost-sharing programme will focus on "the nation's most productive research universities".

Defense Department increases university research support

THE Department of Defense plans to increase by about 25% in 1981 its support of both research and development in general, and basic research in particular, carried out by US universities.

Figures released by the Office of Management and Budget indicate that DOD support for university research is expected to increase only 10.5%, from \$401 million to \$443 million, part of an overall 9.5% increase in federally-funded university research predicted to reach a

total of \$4.6 billion.

However Dr Frank Press told a press briefing that according to Dr William Perry, Defense Under-Secretary for Research and Technology, new programmes planned by the DOD will mean that the actual increase is more than twice this rate. Overall support for basic research by the Defense Department is proposed to increase by 21% in 1981.

Energy research: less for nuclear, more for coal

PRESIDENT Carter is recommending to Congress that the Department of Energy's support for nuclear fission research be reduced by 22% — largely by abandoning work on the Clinch River liquid metal fast breeder reactor — but that there be a 34% increase for research into the uses of coal.

Despite a continued failure to persuade Congress to accept his arguments for killing the Clinch River project, the President is recommending that support for LMFBR research be cut from \$615 million to \$300 million. In addition, he is also suggesting that research be terminated on the gas cooled fast breeder concept, currently supported at a level of \$26 million a year and on the thermal reactor fuel cycle.

Reflecting a growing political interest in coal as a source of energy, the budget recommends coal research be increased from \$779 million to \$1047 million.

Oceanic and Atmospheric Administration.

Funds for basic biomedical research at the National Institutes of Health are scheduled to be raised by 6.2% to \$1.7 billion. Congress is likely to add a little to this figure, but it will be nowhere near as substantial as in the last decade.

Of the various biomedical research institutes, the National Institute of Environmental Health Sciences is slated for the biggest increase — 15% — and the Institute of General Medical Sciences 6%, while the two largest institutes, the National Cancer Institute and the National Heart Lung and Blood Institute, are planned to grow 0.7% and 3.8%.

In the National Science Foundation, which is planned to increase its basic research budget by 18.8%, special emphasis will be given to supporting research in the physical sciences and engineering. For example, the budget request allows for a 16% increase for physics research, 28% for computer research, and 28% for electrical, computer and systems engineering research.

In contrast, support for biological, behavioural and social sciences is scheduled to grow by only 9.3% — scarcely above the expected level of cost inflation.

Prominent in the NSF's budget request is increased funding for research projects jointly sponsored with industry, a move prefigured in the President's innovation

BUDGET REQUEST FOR FISCAL YEAR 1981 (in \$ million)

Agency	R&D obligations ¹			Basic research obligations		
	1981 request	%increase 1980/81	% of total	1981 request	%increase 1980/81	% of total
Defense	16,565	20.2	45.7	523	21.4	10.3
NASA	5,617	9.8 ²	15.6	581	8.3 ³	11.5
Energy	5,106	3.8 ⁴	14.1	593	13.4	11.7
Health and Human Services (NIH)	4,011	6.0	11.1	1,840 1,704	6.5 6.2	36.3 22.6
National Science Foundation	1,056	17.7	3.9	952	16.9	18.8
Agriculture	786	5.8	2.2	324	12.1	6.4
Environment Protection Agency	445	7.3	1.2	19	36	0.4
Interior	415	—1.0	1.1	78	2.6	1.5
Commerce	379	2.2	1.0	36	28.6	0.7
Others	1,756	16.0	4.8	127	39.5	2.5
Total	36,136	13	100	5,074	12.0	100

1. Obligations is the amount of money committed to a programme

2. Takes into account extra \$300 million requested for 1980

3. Excludes cost of spacecraft

4. Excludes energy research money expected from energy security

message to Congress last November. For example, support for small business innovation and industrial technology will grow from \$7.4 million to \$18.2 million, and for industry/university cooperative research from \$7 million to \$20 million.

The most dramatic manifestation of this new linkage is agreement between the NSF and several major oil companies to jointly sponsor a 10-year, \$700 million ocean

margin drilling programme (*Nature* 24 January page 321) using the ship *Glomar Explorer* as a drilling base.

But the Foundation will also be developing a comparable programme of automotive research, which will receive initial federal funding of \$20 million in 1981. The estimated costs of \$1 billion over ten years will be shared between government and the automotive industry. □

Part of these funds will go to three large demonstration projects for coal liquefaction and gasification processes, carried out on a cost-sharing basis with private industry.

\$15 million allocated for new UN science fund

THE Carter administration proposes in its budget request to contribute \$15 million in 1981 to an interim fund to support scientific activities in the Third World agreed at the UN Conference on Science and Technology for Development (UNCSTD) in Vienna last year. The size of the contribution is less than had initially been hoped for, and some feel this may reflect US unhappiness about the reorganisation of science policy machinery within the UN.

High energy physics keeps its head just above water

DESPITE previous agreement that funding for high energy physics would do no more than keep pace with the general rise in costs, the administration has responded to recent complaints by the physics community and agreed to a 10.5% increase in the budget for 1981, officially representing a 1.5% growth above the predicted 9% inflation rate.

This increase will bring the HEP budget

to a total outlay of \$350 million in 1981, a sum which includes the final year of funding for Fermilab's energy saver/doubler project, and the initiation of the tevatron colliding beam facility at the same site.

A 9.5% increase is being requested for nuclear physics. In addition to supporting the continued construction of the National Superconducting Cyclotron Laboratory at Michigan State University, this money will also allow construction work to begin on a tandem accelerator system at the Argonne National Laboratory.

Gamma ray observatory preferred to comet mission

PLANS for a gamma ray observatory were given funding preference by the National Aeronautics and Space Administration over a proposal to initiate work on a joint flight to the comets Halley and Tempel 2 because it offers "a more fundamental mission", according to NASA Administrator Dr Robert Frosch.

The administration is asking Congress for \$19.9 million to begin work on the observatory in 1981, a project actively canvassed for by space scientists for several years as a successor to earlier gamma ray satellites. The observatory is now scheduled to be launched from the space shuttle in 1985.

Dr Frosch told a press briefing that there

was a "reasonably good chance that the much-delayed space shuttle, still officially scheduled for launch this summer, would have its first flight by the end of the year, although there was a likelihood that it would have to be postponed until the first quarter of 1981. In addition to a 1981 budget request \$800 million more than originally planned for the shuttle, NASA is also asking Congress for an extra \$300 million for the current year to cover unexpected costs, largely the result of problems with fixing insulating tiles to the shuttle's surface.

NIH to reduce training grants

THE National Institutes of Health is to reduce the number of training grants for postdoctoral students in 1981 by 7% from the 1980 level of 10,175. The most significant reduction will be in individual grants, which will drop 33% from 1626 to 1080. Institutional grants will be reduced by 2% to 8390. At the same time the value of stipends will be increased by about 5%, and this comes on top of a 30% increase agreed for 1980. In 1981 stipends will range from \$14,040 to \$17,892 a year, compared to \$10,000 to \$13,000 in 1979.

The total value of training grants issued by NIH will be reduced by 7.4%, from \$176 million to \$163 million. At the same time the administration has agreed to keep the number of new competitive research grants at about 5000.

United States

PCP dioxins found to pose health risks

Following wide debate on the hazards of TCDD, US scientists are now expressing concern about the possible health hazards of low-level exposure to other dioxins, particularly those in the widely-used wood preservative pentachlorophenol (PCP).

David Dickson reports

SEVEN years ago, Dale Feucht, a dairy farmer in Canton, Ohio, decided to build a new barn for his herd of several hundred cattle. Two months after the animals had moved into their new home, they began to suffer various ailments, from general ill health to a rapid drop in the calf survival rate.

Veterinarians were at a loss to explain or treat a variety of apparently unrelated symptoms, affecting both existing stock and the animals bought to replace those that died. Within a few years, the herd had been decimated, and the last cattle died last summer.

Having eliminated changes in the water or feed supply as the source of the problem, suspicion focused on the one new factor in the environment, the barn. In particular high levels of pentachlorophenol (PCP), used to treat the wood as a preservative, was found in blood samples from the dead animals — assumed to result from the cattle chewing or rubbing the wood.

Mr Feucht is now suing the barn manufacturers, Morton Company. The case, to be heard in Ohio within the next few weeks, is being watched by many large chemical companies.

For PCP is currently one of the most widespread chemicals in domestic use, particularly in demand for its highly effective anti-fungal and anti-bacterial properties. Over 50 million pounds are produced annually by over 250 companies, and world-wide production is more than 100 million pounds.

Recent events, however, have started to fuel public controversy over the long-term, low level effects of dioxins that have so far been largely overshadowed by the more obvious effects of the most toxic dioxin, tetrachlorobenzo-p-dioxin (TCDD), both a contaminant of the herbicide 2, 4, 5-T, and the chemical released in the Séveso accident in 1976.

The toxic properties of PCP itself have been known for some time. Ironically it was the development of oedema in chicks that first warned scientists of the possible dangers of dioxins. Little is known of the physical, chemical and toxicological properties of other dioxins, particular the hexa-, hepta-, and most prevalently in PCP, the octachlorodibenzo-p-dioxin.

So far, no firm action has been taken against PCPs, which remain on the shelves of hardware stores throughout the country merely with warnings about the need for

careful use. Timber companies, for example, do not issue warnings with wood that has been treated with PCP.

Two years ago, the Environmental Protection Agency, following evidence of the teratogenicity and feto-toxicity of PCP during animal laboratory tests, announced that it was studying whether tougher regulations were needed. These are now expected to be published later in the year.

In the meantime, the lack of strict regulations meant that the Dow Chemical Company, having introduced a new preservative, Dovicide EC7, in which dioxin levels had been reduced two orders of magnitude by using more expensive production techniques, found the product uncompetitive against rival, non-decontaminated brands. Dovicide EC7 has now virtually disappeared from the market place.

But the political momentum previously lacking may now have arrived. Last year residents near the Lexington Blue Grass army depot in Kentucky started to claim that a high local incidence of leukaemia, as well as various other medical disorders, was the result of exposure to PCP used to preserve ammunition boxes at the depot.

Not only had workers in the depot been exposed to the chemical, but wood from the boxes had been sold off for as little as one dollar a car load. This was used primarily for firewood, but investigators subsequently found a range of other uses, including the construction of porches, barns, chicken coops and pigpens.

Following information collected by a local physician, a preliminary study carried out for the EPA by research workers from the University of Miami affirmed that there appeared to be an abnormally high incidence of leukaemia in the area.

Further data are now being collected by researchers from the National Institute of Occupational Safety and Health. They face a double detective task: first to discover whether the leukaemia incidence is substantiated by epidemiological data; secondly to determine whether, given the other substances at the base heavily used during the Vietnam War to store chemical warfare agents, the blame can be placed on the PCP.

But in the wake of the Agent Orange and Séveso incidents, suspicion has been focused on the wood preservative and the contaminants that it contains. And in addition to results of laboratory animal

studies, this suspicion is also supported by recent research carried out at the National Institute of Environmental Health Sciences and North Carolina State University into the effects of PCP in cattle feed.

Research workers at the two institutions conducted a study under which five groups of heifer were compared over a 160-day period. One group was given a feed containing analytical PCP prepared in the laboratory, a second fed with technical PCP containing the dibenzodioxin and dibenzofuran contaminants found in commercial preparations. Two groups were given a mixture of the two. The fifth group was used for control.

The results of the research, which are soon to be published, indicate a number of dose-related effects in the heifers exposed to the contaminated PCP, compared to the controls or those exposed to the analytical PCP. These included a decrease in body weight and feed efficiency, as well as progressive anaemia, and at the end of the test the researchers found a number of unexpected lesions in cattle using the contaminated feed, in particular in the mucosa of the urinary bladder through which the PCP would have been secreted.

"The results of our study show clearly that the toxicity of PCP in cattle is primarily attributable to its contamination with toxic impurities, either the dibenzodioxins or the dibenzofurans," says Dr Eugene McConnell, head of the Comparative Pathology Section of NIEHS and principal investigator of the study.

Which of the two is the culprit has not been demonstrated, but the North Carolina results are broadly compatible with evidence from low level exposure of monkeys to dioxins currently being carried out by Dr James Allen at the University of Wisconsin. Despite its lack of conclusiveness, such scientific evidence is likely to play an important role in forthcoming court cases.

William Brown, the attorney representing Mr Feucht, said last week that he was already dealing with two other PCP-related claims, one from a family that had built a house from PCP-treated timber supplied by a do-it-yourself construction company, the other from a farmer claiming disability after handling PCP-treated posts.

Mr Brown added that he had been contacted by others with similar complaints. A number of other court cases are pending in Michigan. "Everyone is watching this case very closely," says Mr Brown, pointing to the widespread use of PCP-treated timber, not only in homes and farm buildings, but also for railway sleepers and telegraph poles. "I really think it is the tip of the iceberg." □

Soviet Union

Sakharov arrest threatens East-West scientific exchange

Vera Rich reports on the reactions of the scientific community in the West to the arrest of Dr Andrei Sakharov (right) in Moscow last week and his subsequent internal exile



BANISHING Dr Andrei Sakharov, physicist and leader of the human rights movement in the Soviet Union, to Gor'kii is not the way to promote international scientific exchange. This is the clear consensus of the western scientific community.

Referring to Sakharov's "internal exile" as "a shocking" and "a blatantly punitive" act, Dr Philip Handler, President of the US National Academy of Sciences, noted that "in recent years the scientists of both nations have carefully built structures of communication and good will", and that the move against Sakharov "can only be regarded as a challenge to further cooperation and an act of deliberate ill will. . . . I find it difficult to imagine scientific exchange continuing in the spirit we had created heretofore."

"The exile of Professor Sakharov", said Kenneth Boulding, Chairman of the Board of Directors of the American Association for the Advancement of Science, and Frederick Mosteller, President of the AAAS, "deprives the people of the Soviet Union and the world of a brilliant voice in support of mutual understanding and the defense of human freedom". His banishment, according to 25 US Nobel laureates, who sent their own personal telegram to Brezhnev "will silence an eloquent voice of wisdom desperately needed in our troubled world".

For the US Committee of Concerned Scientists, Drs Max Gottesman and Mark Kac, expressed their concern that the move against Sakharov coming "as the aftermath of Afghanistan" might "portend a change which threatens the continuance of cultural and scientific relations between our two countries." Telegrams from the French Académie des Sciences and Société des Physiciens expressed similar shock and concern.

In London, the "President and Officers" of the Institute of Physics, expressed "shock and dismay" fearing that "this action . . . will damage both Soviet physics and the relations between Soviet and British physicists". The Royal Society, with its usual diplomacy, expressed the view that "voices sound louder through doors that are open than through those that are locked".

This is not the first time that western colleagues have felt obliged to leap to Sakharov's defence. In September, 1973, an ominous *Pravda* article, entitled "To be a Russian scientist means to be a patriot"

was widely seen as a first move towards expelling Sakharov from the Academy of Sciences. No expulsion in fact took place, perhaps due to the storm of foreign protests the article provoked, but also to the reluctance of the Soviet Academy to permit undue government and Party meddling in its internal affairs. (The Academy is the last remaining Soviet body to have elections by secret ballot with a plurality of candidates). A short while later, when a similar attempt was made to deprive Veniamin Levich, the Jewish "refusnik" of his "Corresponding" (i.e. Associate) membership of the Academy, the attempt failed, his son Aleksandr told *Nature*, "because even some members who were quite anti-Semitic had decided to vote against the motion, feeling that their own position was threatened by any attack on the Academy".

Perhaps as a pre-emptive measure against such academic solidarity, Soviet "explanations" of the Sakharov affair have stressed how much he is out of step with the ideal of a Soviet scientist. The initial allegation (in *Izvestiya*) was that he had abandoned science for "the political walk of life" following a crisis in his own creativity. (No mention was made of his sudden dismissal from his government job — following the *samizdat* publication in 1968 of his "thoughts on Progress, Coexistence and Intellectual Freedom").

This approach, however, was negated when the western media stressed that Sakharov had been arrested on his way to a physics seminar at the Academy of Sciences — and by confirmation from close friends that he was still, in spite of the demands on his time made by his human rights activities, keeping up his theoretical work on physics, though with little hope of publication. Indeed, the *Izvestiya* accusation, that Sakharov was being "used as a channel for passing on important Soviet secrets to the . . . imperialist powers" was incongruous with their allegation that he had abandoned science.

The next stage came with an attack on Sakharov by individual members of the Academy. Academician Evgenii Fedorov spoke of the commitment of Soviet scientists to "social work, the struggle for peace, cooperation with our colleagues abroad in science and technology" (almost echoing the title of Sakharov's first essay!). A "large community" of Soviet scientists, he said, are "fed up with Sakharov's

remarks and his general attitude . . . contrary to everything for which Soviet people, including representatives of Soviet science, have fought". Academician Mark Mitin spoke of the "humanitarian" action of the Soviet government, which in spite of Sakharov's slanders of the Soviet way of life had simply deprived him of his state honours and sent him out of the capital. Nevertheless, Mitin observed unctuously, "as a member of the same Academy of Sciences I cannot but feel sorry about the downfall of this man."

Some, it would seem, felt more than mere sorrow. The resignation of Vladimir Kirillin from his posts as Deputy Prime Minister and head of the State Committee for Science and Technology was almost certainly connected with the Sakharov affair — though speculations vary as to whether Kirillin resigned voluntarily in protest or was manoeuvred into a situation where resignation became inevitable. Of recent years he has been a well known visitor to Britain to negotiate extensions to exchange agreements in technology and trade. (The next round of talks, due in London in May, will almost certainly now be at least postponed).

During the past few months, there have been a number of indications of conflict between the State committee (which comes directly under the government) and the Academy, which still shows a remarkable independence of spirit, particularly where its own autonomy is concerned.

Although the move against Sakharov must be seen against a whole wave of repressions against a wide spectrum of human rights activists over the past few months, the fact that the authorities felt able to renew their challenge to the Academy may suggest that the strength of the Academy has somewhat waned in comparison with 1973. Certainly, it is Dzherman Gvishiani, a representative of the State Committee who will lead the Soviet delegation to next month's Hamburg Forum — an event which, incidentally is still going ahead, although as one of the organisers told *Nature* "current political tensions will naturally be reflected there in some way". □

Soviet Union

Fire threatened fast reactor cooling system, says unofficial report

"We have no accidents which would give rise to anxiety among people and set them against the development of nuclear power engineering," Andronik Petrosyants, chairman of the State Committee for the Utilisation of Atomic Energy, told the press recently.

This, however, is not the opinion of the townspeople of Sverdlovsk, in the Urals. A clandestine report which has just reached the West gives a vivid description of the alarm caused by a fire at the Beloyarsk nuclear power station some 60 km away.

The author, who identifies himself only as "N.N." appears to have inside knowledge of the workings of the power station. He alleges that during New Year's Eve celebrations in 1978/79, a fire started in the "machine hall". Several firefighters lost their lives in combatting the blaze. It was not brought under control until "everything that could possibly burn was completely burned up."

"N.N." admits that there was no immediate risk of the reactor exploding; however, the reactor cooling system was, he says, threatened. The Beloyarsk station has a fast-reactor, and the primary circuit of the heat-exchanger contains highly radioactive molten sodium at 500°. A rupture in the primary circuit would cause an explosion scattering radioactive material in a cloud like that produced by the 1957 Kyshtym disaster.

"N.N." says that the threat of a similar cloud from Beloyarsk was sufficiently real for the authorities to provide special trains and buses, standing by to evacuate, if necessary, the settlement of Zarechnyi close to the plant.

Following the fire, says "N.N.", a KGB team from Moscow, led by a General, came down to investigate. Their findings remain, he says, unknown, but the local population feel that a significant part was played by the fact that "the shift was drunk, like almost everyone else in the Soviet Union that night." No official allusion has been made so far to any fire at Beloyarsk.

Like the 1973 fire at the Shevchenko fast breeder, which was due to a rupture in the sodium circuit, the Beloyarsk fire was not technically a "nuclear" disaster. According to Academician Anatolii Aleksandrov, President of the Soviet Academy of Sciences and himself an experienced nuclear engineer, fully half the funding of nuclear power stations is spent on safety.

This however, N.N.'s report suggests, is insufficient; the human factor also must be taken into account.

Vera Rich

High energy physics

End of the physics phoney war

Competition for the prize of the intermediate vector boson is leading to doubts about the future of CERN, the European centre for subnuclear physics. But the real competition may be trans-Atlantic. Robert Walgate reports

In a few weeks' time, the European Committee for Future Accelerators will be holding an extraordinary meeting to discuss what amounts to a perceived competition between West Germany and CERN. ECFA, which has no constitutional power but which represents the views of an influential body of European physicists, is thus cautiously falling in line with Italy, which first shed doubt on Germany's intentions.

The Chairman of ECFA, French physicist Professor Marcel Vivargent, was recently quoted in an Italian newspaper as saying that Italian efforts to clarify CERN's future programme "merits the best attention" and is supported by many European physicists. (In late December, the Italian delegation to CERN walked out of a meeting of CERN Council in a protest at "lack of sufficient discussion" of CERN's future, and allusion to potential German competition.)

Vivargent told *Nature* that he understood the Italian position, as a relatively small nation relying on CERN: "they want CERN to remain the top lab in Europe". He also said that he understood the German desire to find a future for its lab, DESY. The problem, which had been neglected by CERN Council, was to mesh the two objectives together.

Strictly ECFA will be discussing 'complementarity' between two accelerator proposals; HERA for the German laboratory DESY near Hamburg, and LEP for CERN. HERA is designed ultimately to collide 30 GeV electrons with 820 GeV protons and shed light on interquark forces, and perhaps the structure of the quark. (It is equivalent to a 52 TeV electron linac firing at a stationary target, giving a 'spatial' resolution of some 100,000th part of the proton radius.)

But the first part of HERA, according to its coordinator Bjorn Wiik, will collide 35

GeV electrons with equal energy positrons. It will operate in this mode for four years before the proton ring is complete. "Our proton ring will be by far the biggest superconducting effort in Europe" explains Wiik "so we have allowed a long lead time to build it". According to present targets, the electron phase of HERA will be ready in 1985, and the protons in 1989.

LEP, on the other hand, is dedicated to the collision of electrons and positrons at beam energies between 22 and 130 GeV. Protons do not come into the proposal, although the proximity of the CERN complex of proton machines, up to the 400 GeV SPS, make the use of protons a possibility.

The gem in this energy range is the Z^0 , which at 90 GeV would be the heaviest particle yet and a key element in the unified theory of the weak and electromagnetic interactions. In the theory, due to Abdus Salam and Steven Weinberg, the Z^0 plays the role of the photon in the weak interaction (which causes nuclear decay and limits the output of the stars).

The Z^0 (or neutral intermediate vector boson) is mesmerising physicists like Moby Dick mesmerised Captain Ahab. LEP would create it (if it exists) with 45 GeV colliding beams. So would HERA, if it could be stretched up to that energy; and since approval for LEP is unlikely to come before mid-1981, and construction (at a constant CERN budget) is predicted to take seven years, there is a possibility — it is argued — that HERA would have snatched the Z^0 four years before LEP.

The question is actually more complicated, because CERN is likely to find the particle in 1981 — but not with LEP. CERN is building a proton-antiproton collider which will produce 270 GeV on 270 GeV collisions towards the end of 1981, with a view to creating a few Z^0 s. But it will not produce many — opinions

Machines which might compete for the Z^0

Device	Projected cost	Circumference	Earliest likely physics	Beam energy	Luminosity**
LEP	\$640m	30.6 km	1989***	22-130 GeV**	$10^{32} \text{cm}^{-2}\text{s}^{-1}$
HERA	\$167m++	6.5 km	1985 (electrons)	35 GeV	10^{32}
SPC	\$60m	1.8 km*	1984	50 GeV	10^{30}
antiprotons (budgeted)		6.8 km+	1981	270 GeV	$10^{30}++$

* in two loops

+ in the SPS ring

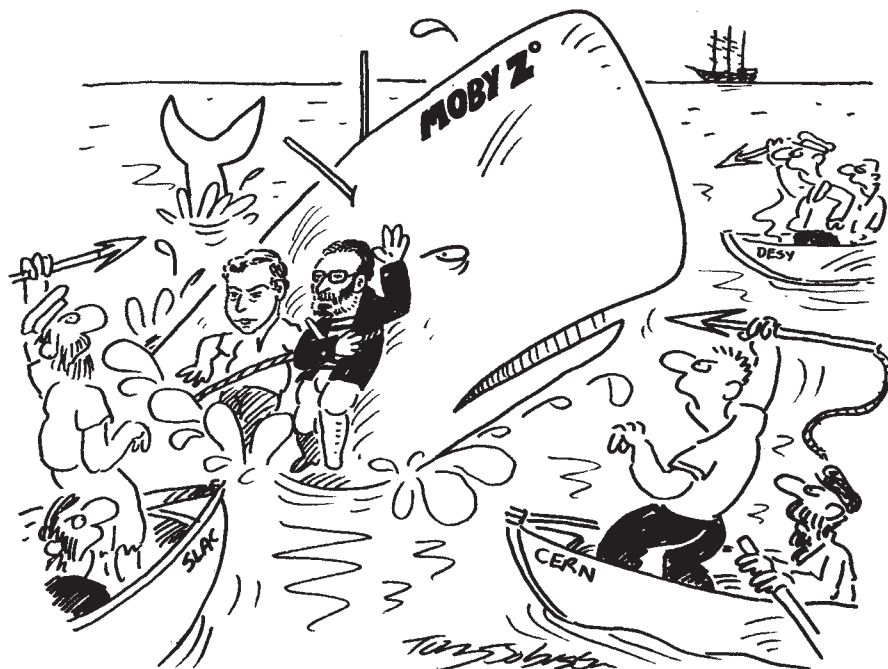
** main option 86 GeV

+++ electrons only; with protons double this

** event rate per unit collision cross-section at peak energy

*** fraction of events producing Z^0 some 30 to 50 times lower

+++ could be earlier if large fraction of present research programme cut



differ, but perhaps some four orders of magnitude fewer than LEP — and those will come among a great deal of unwanted matter (from the spare quarks in the proton and antiproton).

The goal is to produce a lot of clean Z^0 s, so that they can be studied in detail. If HERA stole LEP's thunder, questions would be asked about the value of LEP. And since CERN has argued that it can run down a number of its facilities, such as the intersecting storage rings, to leave room in its present budget to build LEP, it may find that member states are asking it to run down — without LEP.

But Wiik, HERA's builder, quashes the speculation. "At 45 GeV, the synchrotron radiation [X- and gamma-rays emitted as the electrons turn corners] in HERA might be completely prohibitive" he told *Nature*. "And we'd need a large investment in extra cavities — something we haven't budgeted for".

The principle limitation on the energy of electron machines is the amount of radio-frequency power that can be pumped in to keep the electrons in their orbits, to compensate for synchrotron emission. The field of the bending magnets is usually low, and not a limitation (contrasting with proton machines).

"Furthermore" says Wiik "the geometry of the machine is not optimised for 45 GeV". DESY has designed in long straight sections (which increases corner curvature and hence synchrotron radiation) to allow polarisation of the electrons for electron-proton studies. Wiik could not give a figure for the power consumption that HERA would demand at 45 GeV (although it increases from 8 to 13 MW from 30 to 35 GeV) but added that "I don't know even if we would have room to put the extra cavities in". According to one observer, superconducting cavities would be necessary, and these are not likely to be

available by 1985.

The HERA design is almost complete; and later this year there will be an application to Bonn for the DESY budget to be increased by some DM60 million a year from its present DM150 million. Over 10 years, this would be expected to pay for both phases of the accelerator; there will not be a formal application to build HERA. The CERN subscription would not be affected either; "that money comes from a different pot" said a DESY spokesman.

Competition for LEP, however, may arise from an entirely different quarter: the United States.

Over on the West Coast, at the Stanford Linear Accelerator Center, Professor Burton Richter has a plan for a cheap " Z^0 factory". At an estimated \$60 million ("plus or minus 25%" says Richter) the 'single pass collider' would undercut first phase HERA costs by a factor of three, and LEP (main 86 GeV version) by a factor of ten.

Richter's device gambles on the presence of the Z^0 (a fair bet) and makes use of the 90 GeV resonance to avoid the need for large and costly storage rings. Thus Richter turns the tables. His small storage ring SPEAR won him the Nobel prize and set the current fashion for storage ring physics (LEP is basically a scaled-up version of SPEAR).

Since the cross-section at the resonance will be very high, he reasoned, a single collision between one bunch of electrons and one of positrons should be enough to produce a reasonable number of events. So he constructed a plan around the SLAC one-mile electron linac that would do the job. It involves increasing the energy of the accelerator to 50 GeV; and accelerating an intense pulse of positrons down the linac followed, 20m behind, by one of electrons. At the end of the accelerator these pulses are wheeled around a radius of 300m one to

the left and one to the right, and brought into collision.

Richter is very confident that the machine can be built, despite very close tolerances. SLAC is pursuing a design study and will make a request in March for full funding. If the application went smoothly, the SPC could begin building in October 1981, "but I give that no better than evens" said Richter. In the long term he considers political prospects for the collider to be "very good. Everyone is enthusiastic about it".

Since the project is small enough to avoid the need for an externally imposed managing architect (the choice of which delayed PEP, SLAC's medium energy electron ring, due to start in March, by nine months), the SPC could be built in two and a half to three years, thinks Richter.

The technical obstacles are: accelerator improvements, where SLAC is putting in its largest theoretical effort (it will be necessary to align the accelerator components to 0.3mm to stop the long pulses slewing around, tail to the side) increasing the brightness of the positron source by a factor of two (a design exists for a factor ten, but engineering R&D is necessary); "damping" the energy spread of the positrons from the source; synchrotron radiation in the 300 m bends; and a final focus of the beam spots to 2 μ m.

This latter compares with the 100 μ m $\times$ 1000 μ m spot in SPEAR, or the 90m diameter spot emerging from the linac. But the exercise is one of geometrical optics, particularly getting rid of the 'chromatic' aberrations present in the beam transport system given a beam momentum spread of 0.5% and this is designed in principle.

Finally the question is the number of Z^0 that the SPC could produce. "A million a year" says Richter — quite enough to learn a lot of physics. LEP would have 100 times more, and eight experimental regions, but the SPC ought to be able to detect all but the rarest decay modes of the particle.

This leaves LEP with the rare decay modes, and its higher peak energy, which with its main (86 GeV on 86 GeV) version should produce the charged companions to the Z^0 , the W^+ and W^- , which are produced most efficiently in pairs.

This is good physics, but there are some doubts about how efficiently a storage ring could work at this energy. Further the power consumption will be extremely high (some 250 MW at peak power). LEP will consume an average 750 GW hours per year for 3,000 hours of experiments at 85 GeV raising doubts about running costs against electricity costs in the late 1980s and 1990s.

Although physicists are in wide agreement that LEP is by far the best possible machine to explore the physics of the Z^0 and the W s, and to search out what lies beyond Weinberg and Salam, the machine is strangely beginning to look like a Rolls Royce against competition of a Model-T Ford which, nevertheless, gets you where you want to be. □

NEWS IN BRIEF

Livermore earthquake raises safety concerns

A debate about the safety of nuclear research facilities at the University of California's Lawrence Livermore Laboratories has been rekindled by an earthquake which hit the laboratory last Thursday causing minor injuries to almost 30 employees, and leakage in a storage tank containing tritium contaminated water. According to the laboratory, no radioactive material was released into the atmosphere or the environment as a result of the accident and key research buildings containing plutonium and tritium sources were undamaged. However, the laboratory added that a 30,000 gallon tank containing 50 microcuries/cc of tritium had leaked about 50 gallons into an asphalt basin on the ground, although none had escaped from the containment building.

The earthquake is also reported to have caused \$1 million worth of damage to the Shiva facility. The bolts, holding down the metal frame holding the 132 ft facility, sheared. Other damage at the laboratory included the displacement of filing cabinets and office trailers which were knocked off their stands. Last year, the earthquake hazards faced by the laboratory became a major source of controversy when local groups seeking to curtail nuclear weapons research at the laboratory claimed that earthquake damage could lead to a major leak of radioactivity. Last week's earthquake which measured 5.5 on the Richter scale and was centered 10 miles from Livermore was one of the severest to hit the San Francisco region since the major earthquake of 1906.

Plan to dump toxic wastes in Third World

A number of US waste disposal companies are drawing up plans to dispose of toxic wastes in Third World countries as a result of the imminent introduction of strict regulations covering the dumping of such substances by the Environmental Protection Agency.

One Colorado company was reported last week to have plans to open waste recycling plants in three foreign countries, thought to be Sierra Leone, Liberia and Chile. Officials from the company, Nedlog Technology Group Inc., said that the plants are planned to extract metals such as copper, cadmium and zinc from industrial wastes.

Under new regulations it might prove impossible to dispose of wastes from this process in the US. The company was therefore "looking overseas to find countries that need foreign exchange and jobs and have the plant sites," said company vice-president James R. Wolfe.

State Department officials, however, are

worried that any such moves by US corporations could generate a backlash in the developing countries, which might decide jointly to "condemn the US for dumping its wastes in the black man's backyard".

Deutch to resign from Energy Department

THE US Department of Energy confirmed last week that Dr John Deutch, previously director of the Office of Energy Research, and currently Under-Secretary of Energy — the third ranking position in the department — had submitted his resignation to the White House.

During his nomination hearings before the US Senate in 1977, Deutch made it clear that he intended to remain in Washington only for a limited period of time before returning to his position as professor of chemistry at the Massachusetts Institute of Technology.

However, speculation about Dr Deutch's future plans have been raised by reports that he may join President Carter's re-election campaign as an energy adviser and that White House aides are said to be increasingly disappointed with the political performance of Energy Secretary Charles Duncan. In particular, they are critical of his failure to "politicise" the department, for example by not taking a strong public stand criticising Senator Edward Kennedy's opposition to the decontrol of oil prices.

Nobel Prize changes Pakistan's science

Science in Pakistan has received a major boost after the award of the 1979 Nobel Prize for physics to Abdus Salam. Pakistan's President, General Muhammad Zia-ul-Haq has publicly promised to allocate 1% of the country's GNP for scientific and technological research in accordance with suggestions made by Salam. This would mean an expenditure of \$100 million annually instead of only \$20 million at present.

The President has also agreed to give serious consideration to another far reaching proposal made by Salam at the Special Convocation held at the University of Islamabad during his recent two week visit to Pakistan. That is to make science, like religion, a compulsory subject at all levels of education. Since Salam's visit, scientists have been made exempt from the general ban on travel abroad.

Azim Kidwai

French cracks cause new nuclear start up delay

THE French Ministry of Industry announced last week that it would

postpone by "several weeks" the scheduled start up of the troubled nuclear reactors, Gravelines I and Tricastin I. Cracks in the tubular base plates of the heat exchangers in the primary cooling circuits have already caused delays because of strike action by the trade unions involved who have demanded additional safety measures. The new delay will permit further tests to be made on equipment that has been designed to follow automatically the evolution of the cracks during the lifetime of the reactors. Preliminary tests of the monitoring equipment by the manufacturer, Framatome, have been inconclusive because they have been done on surplus plates, originally scheduled for export to Iran, that have no cracks.

In the meantime, a petition campaign launched last June by 22 organisations for "an alternative energy policy and for a democratic debate on energy" has received 500,000 signatures.

Ariane given to private industry

THE council of the European Space Agency made final last week the French proposal to transfer the Agency's launch capability to the private firm Ariane-Espace. Ariane-Espace will be responsible for production and launching of all future ESA satellites. It will charge the Agency the following prices for various kinds of launches: 175mFFr for a launch requiring full Ariane power, 150mFFr for a satellite launch requiring only part power, and 95mFFr for a satellite that can be launched along with another in a double payload. The director-general of ESA will engage in discussions with the directors of Ariane-Espace to elaborate the details of the arrangement.

The ESA council also voted approval of the delayed 1980 budget and made the Earthnet programme of earth and meteorological satellite observations part of the official ESA budget. Earthnet had been previously funded on a voluntary basis by member states. The council also voted the funds for the Spacelab programme. In a related development, Switzerland, which is not an ESA member state, voted a sum of 7mFFr to participate in future improvements of the Ariane launcher.

Correction

In *Nature* 17 January, page 237, the statement by the Scientific and Medical Committee for Soviet Jewry should read: "not only should the Hamburg meeting include on its agenda the question of the freedom of movement of scientists, also any scientific body that may arise from the meeting must ensure that a clause dealing with this problem is written into its constitution".

FEATURES

China: where the new and the historical materialism interact

Although scientists in China are being forced into a new role by rapid changes in Chinese life and closer contacts with the West, they are still guided by Marxist philosophy and the principle of social relevance. In the first of three articles on developments in Chinese science policy over the past year, **Tong B. Tang**, describes the organisation of research. In future articles, he will discuss higher education and the popularisation of science.

SCIENCE in China is growing increasingly quickly. It is based on ancient scientific heritage, the tradition developed under Marxism over the past 30-60 years, and the critical adoption of western methods and ideas. The importance of the last factor, however, has yet to be assessed. Although China stresses that its science will take a different path from that of western countries, its science and technology now seem poised to fill the economic and military roles which they have long occupied in industrialised countries.

China needs to promote the national economy and to deter what it calls international and regional hegemonists with greater help from the modernisation of science. Hence, the direction and prospect of its science are bound to be of profound interest, not only to scientists and science historians, but for the world.

Most scientific research is carried out at the 112 institutes of the Academia Sinica and at the 600 or more universities. The Academia, which celebrated its 30th anniversary in November 1979, is the designated "comprehensive centre of research into the natural sciences".

The entire pure and applied science

budget is administered by the State Scientific and Technological Commission with Vice-Premier Fang Yi in charge. The Commission's preferences for different disciplines basically follow the National Plan for the Development of Science and Technology, a document of many volumes which was collectively agreed at the All-Nation Science Conference in March 1978 (*Nature*, 6 April 1978, page 482). Briefly the orientation is towards agronomy and applied physics. The Plan was slightly modified when the three years of 1979-81 were recognised as an adjustment period for the national economy, and a conference was convened by the Commission in November 1979 to discuss the implementation of these modifications, particularly in the area of science and technology transfer.

The Commission allocates research responsibilities and funds to the Academia or the Education Ministry, and then successively to institutes or universities, sections or faculties, each represented by 'academic committees', and finally research groups. The Academia, nominally led also by Fang Yi, has Li Chang as its First Vice-President, and the Minister of

Vice Premier Fang Yi, in charge of China's science budget and head of the Academia Sinica, meets students in Beijing



Giant propaganda poster stresses the political context of modernisation

Education is now Jiang Nanxiang. Key installations may be operated directly by the Academia or the Ministry or both. In addition, other ministries may support researchers whose work is of direct relevance to agriculture, forestry or industry. Some of them run their own chains of research institutes and training colleges. However, the infrastructure that couples the (long-term) activities of institutes and universities to research carried out at scientific farms and research and development at factories appears weak; one indicator is the relatively long delay that sometimes occurs between completion of a project and the application of its result in production.

Most research projects are assigned from above, but occasionally a research group submits a proposal to the academic committee of its section or department, whose complete programme will be reviewed by the institute or university. But it is unusual for a research group to branch out in its own research areas. This contrasts with western universities but probably resembles more the situation in government and most industrial laboratories. Last November, however, the Academia passed a resolution to give institutes the freedom of securing external funding by taking on contract work. The research unit will be able, after making sure that its state-assigned projects will be

Tong B. Tang is a research fellow with the physics and chemistry of solids group at the Cavendish Laboratory, Cambridge

completed on time, work on problems offered by a factory. Its time and expenses will be repaid by the factory either in one lump sum or as a percentage on the sale profit of the product concerned. It does not have to hand back to the state any net income that may result, but this procedure is said to be temporary only.

That the applied sciences continue to dominate in China is a reflection of the conviction that science receives its impetus from social needs: "from the very beginning the origin and development of the sciences have been determined by production". However the "socialist newborn-thing" of "inviting in" workers and peasants with practical experience to participate in experiments, and to a lesser extent that of scientists "going out" to work with the masses have largely gone. Now more 'pure' research is being encouraged. For example, two groups, one in Beijing, the other in Guangzhou, have started work on the detection of gravitational waves, and an observatory is planned near Kuming to carry out a comprehensive survey of the southern sky. More well known, perhaps, is the construction of a 50 GeV proton synchrotron at Beijing (the 'BPS').

Opinion is divided on the social relevance of going in for high energy physics in a big way, especially among guest advisors such as the Nobel prize winners Chen-Ning Yang and Tsung-Dao Lee. But Li Chang explained at the All-Nation Science Conference that particle physics, being a frontier subject, would play a leading role by stimulating advances in instrument technology and other fields. (The other two 'frontier sciences' which have been identified in a National Conference on Basic Sciences Planning held in October 1977 are astrophysics and cosmology, and the origin of life.) Fang Yi recently reiterated: "We consider basic research fundamental to all scientific progress".

Unfortunately, at present there is probably much duplication of applied research effort, on, for example, metal oxide semiconductor interfaces, stacking faults on oxidised silicon and other topics related to integrated circuit technology, rare earth metals as catalysts in petroleum refining, and lasers. All of these are among the priorities specified in the 1978-85 development plan. However, the nationwide coordination of research and resources and the exchange of results are hampered by bureaucratic barriers between ministries and administrative bodies, although the bureaucracy is no worse than in the West.

The Chinese are also discussing the relationship between central planning and local initiative. There is a trend for political authorities at the provincial or municipal level to take a greater part in the administration of science. Provided that democratic centralism is not eroded, and that the relationship of local autonomy to



Worker-technicians Tsao Mu-Chuan and Chang Shih, working with China's first electron microscope

central control follows the changing situation, a limited devolution should make science more responsive to local needs without hindering the fulfilment of a unified state plan. Less tangible, though, are certain ideas on a 'mixed' system for research management, namely that science grows most quickly when it is also partly based on 'market economy' principles.

Many measures are still of a tentative nature. With some problems a variety of different solutions are currently being explored; a common solution will be nationally implemented only after an appraisal or reappraisal of all relevant experience. Up to the winter of 1978-79, policy statements were inclined to be contentiously forthright, for example, those concerning material incentives, learning from the West, and the professionalisation of education. Some of them were more 'liberal' than in the years immediately before the Cultural Revolution, even than in the mid-1950s. Since spring of last year this tendency has been reversed. For instance, there has been more propaganda in the media and on public displays emphasising political work, revolutionary heroism, self-reliance and the danger of examination worship.

Western scientists in general and those of the social disciplines in particular are enthusiastic about the burgeoning academic exchanges with China; about these more will be said in the next article. The Chinese welcome these developments but have firmly concluded that there is no question of importing wholesale modernisation or science and technology. The current slogan concerning foreign technology is, "use, learn, then modify, and finally invent". In April last year a

meeting of academics in Beijing urged that Chinese science should retain its original character. At the First National Symposium on the Science of Science in August 1979, Li Chang reaffirmed that China will nurture a modern science, different from western science, by integrating the latter with the "dialectics of nature". Some Chinese have claimed that the dominant methodology endorsed by western science, while materialistic, is "metaphysical". This means that it contradicts the dialectical laws of thought, with its implicit assumptions of irreducible isolates and unitary identities.

Many events confirm that the prominent features of Chinese science in Mao's era have remained intact and are being further enriched. The Chinese understanding of science (and arts) as orientated towards man's struggle in economic production comes from Marxist historical materialism. Efforts to combine the modern and the traditional, initiated as part of Mao's policy of "walking on two legs", continue. For instance, the Peking College of Traditional Medicine is among the five key medical schools named by the Education Ministry, and traditional medicine is on the (essentially standardised) curricula of all fourth-year students training for western clinical practice or research. Philosophical arguments based on Marxism retain a respected guiding position in scientific controversies. For example in particle physics the quark model is overwhelmingly preferred to the bootstrap hypothesis of hadrons, and the high-sounding applications of catastrophe theory to human phenomena are generally ridiculed.

Further, guided by Mao's conceptions of "one divides into two" and the transformation of opposites, the Chinese want science to "change wastes into wealth and turn harmful into beneficial" in all agricultural and industrial processes. Lastly, to achieve modernisation and a healthy socialisation of science, the Party pledges to mobilise the whole people, in accordance with the dictum that "the mass movement is necessary in all work. Things won't go without the mass movement".

Nevertheless, in its quest for rapid modernisation, the Party has broadened the political base of decision-making and given more scope to the initiative of experts. Senior scientists now join forces with state officials to administer science and education. The press has devoted considerable space to polemics on democracy in academic discussions, or "academic freedom", meaning that the minority can sometimes be right and should always have the right to disagree. Indeed, the frustration of scientists may be understandable especially if it arises from the indifference or the dogmatism of bureaucrats. Still, the leadership will not want greater freedom to result in wasteful competition, an erosion of planned research or the ascent of deviationists and technocrats. □

Energy: the need for technical fixes

Model-based predictions of energy demand and supply are unreliable, since the behaviour of individual producers and consumers cannot be guaranteed. **Alvin M. Weinberg** (right) of the Institute for Energy Analysis at the Oak Ridge Associated Universities, Tennessee, argues that technical fixes involving increased supply offer the most reliable way of averting an energy crisis

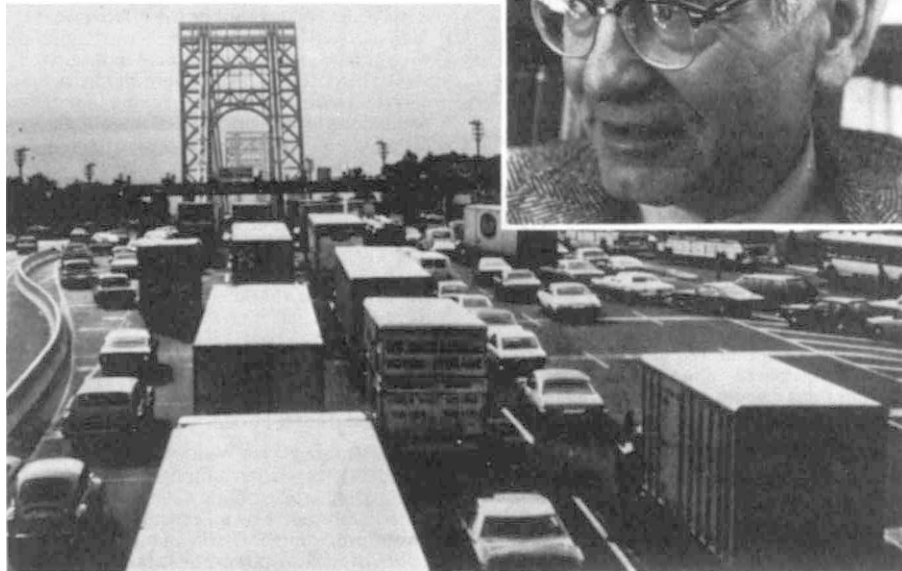
I first became acquainted with energy demand modelling when I worked for the Federal Energy Administration in 1974. President Nixon had announced Project Independence: the United States was to import no oil after 1980. The FEA's job was to figure out how to reach this goal, or at least some goal that was consistent in spirit if not in fact with the President's announcement. For this purpose, it set up the most elaborate model the budding art of energy modelling had yet seen. Many millions of dollars were chewed up on two CDC 7500s; their neatly printed graphs showed how energy would be used and how it would be supplied in the United States through the year 1985.

A prime question was the price level of imported oil at which one could expect domestic supply to be so enhanced, and demand so dampened, as to reach the goal of self-sufficiency. The computers gave a fairly clear answer. At \$16 per barrel, \$6 per barrel higher than the 1974 OPEC price (at 1979 values), oil imports could fall to zero by 1985, provided the government encouraged accelerated production of domestic oil. The model predicted that, even without government intervention, US imports in 1980 would be only about 6 million barrels per day, falling to about 5 million barrels per day in 1985. As things turned out, the US imported more than 8 million barrels per day in 1979 even with the price of imported oil a good bit higher than \$16 per barrel.

Looking back, it is apparent that the FEA model had overestimated the demand elasticity for liquid fuel — the percentage decrease in demand for a given increase in price. The output of the entire model depended critically on the value of this elasticity. Demand elasticities are notoriously uncertain: those used in various models differ by a factor of ten!

Last October, I (along with 200 energy modellers) attended the Dublin meeting on Energy Systems Analysis sponsored by the European Economic Community. The EEC is sponsoring a "super" model that encompasses the entire community; with sub-models applied to each country. At the Dublin meeting the modellers could compare the results of application of the EEC methods to each country.

This effort is of extraordinary scope. Yet, as the modellers themselves conceded, one can hardly depend on its predictions. Unexpected things happen. The Shah is



Increased oil prices have not deterred US consumers, so Carter has chosen to develop synfuels

overthrown; people simply don't react the way the models assume they should.

The moral is clear: policymakers must treat models warily. This is hardly an original view. Yet it seems that the modellers in the United States have had remarkable success with the opinion-makers if not, as yet, with the policymakers.

Most of the more recent models tend to stress reduction in demand rather than increase in supply. This viewpoint is promulgated in the recent report of the Harvard Energy Project, *Energy Future*. The book has already sold more than 100,000 copies. It has been widely acclaimed in the American press as containing both the best appraisal of the energy problem, and the best strategy for its resolution.

To meet the US demand in the late 1980s, the Harvard study estimates that 8 million daily barrels of oil equivalent (mboe) can be saved by conservation, and 3 mboe by widespread use of solar energy. "Conventional" supply, meaning coal and nuclear, is increased by only 5 mboe. These numbers are to be compared with the 37 mboe equivalent total US demand in 1977. Conservation and solar energy, by the late 1980s, are supposed to take care of almost one-third as much energy as was supplied by all energy sources in 1977. Although the Harvard study properly warns that conservation and a transition to 'Solar America' depend on innumerable

decisions made by countless people, all conserving energy or installing their own solar devices, it leaves the impression that here was a true, and ideologically acceptable, path to energy salvation.

By contrast, Jimmy Carter's response to the energy situation last June was, to a remarkable degree, supply-oriented: spend \$80 thousand million for several million barrels per day of synfuel. The President chose to put most of his money on a technical fix: synfuels. How much of the President's proposal will survive in Congress will depend on how much political pressure is generated by those who believe conservation and solar energy come painlessly.

I doubt that the President and his advisers weighed the uncertainties in predictions of demand when they decided to go for increased supply. But I believe their instinct was right. Technical fixes, especially those involving increased supply, require decisions by relatively few people. They finesse the impossibility of knowing how and how quickly 220 million people will react to a change in price.

Obviously the proper course is to increase supply as well as to conserve. This piece of common wisdom seems to be ignored by some of our noisier conservationists. Despite opposition from this segment of the energy community, I hope the President's commitment to augmenting supply will receive the public support it needs to survive in Congress. □

CORRESPONDENCE

French nuclear cracks pose no danger

SIR, — The article entitled "France faces a cold winter" (29 November, page 436), states that: "Although these cracks are not dangerous at this stage, Framatome experiments show that they tend to enlarge under the temperature transients of normal operating conditions, leading to possible component failure within four to six years, a failure which clearly would have catastrophic consequences involving the total loss of primary coolant."

And further that: "The only other possibility is permanent shutdown after five years" and: "These were the facts made public by both government and unions."

We regret that *Nature* did not consult the manufacturer of the components on which the cracks were found. The time limit of four to six years mentioned in your article (the currently accepted figure is six to eight years) concerns the minimum time for the cracks to fully penetrate the 10 to 16 mm thick stainless steel cladding which separates the reactor coolant from the component perse, and *not* the minimum time for component failure. Moreover this minimum time results from calculations based on the most pessimistic assumptions derived from experimental evidence. Similarly pessimistic calculations show that, after the cladding has been fully penetrated, component penetration could only occur several decades later. Furthermore, such penetration would result in a slow leak of primary coolant and in no way lead to what your article describes as "catastrophic consequences involving the total loss of primary coolant".

As concerns the last paragraph of the article, we have never been informed of any statements which contradict our position.

Yours faithfully,

JACQUES GAUSSENS

Framatome, Paris, France

The technical experts must be heard

SIR, — In your issue of 3 January (page 1) you had a short editorial entitled "Nuclear power: the critics must be heard". This seemed to me entirely sound but it is only half the story. Surely it is at least as important that the technical experts should be heard, and indeed listened to?

It is of course possible that the experts are biased, but it is also possible that critics are biased and while senior engineers might be unwilling to disclose all the relevant facts, you do not become a senior engineer by either ignorance or misrepresentation of the technical facts of your profession and it is likely that the facts presented by such people are correct and worth listening to even if it is fair and reasonable for the critics to be exploring for facts which have not been released.

It must be apparent to anyone who has been watching the field that the media, especially the broadcasting media, frequently present as facts some quite wild guesses by critics and not infrequently fail to present facts given by the other side at all. A particularly serious example of this was a BBC programme towards the end of last year in which Dr Alice Stewart was given a sensible amount of time to discuss the reasons why she believes that doses of radiation are some 20 times as likely to produce cancer as is expected by the International Commission for Radiological Protection.

In a long taped interview Dr Reissland of

the National Radiological Protection Board gave a number of detailed reasons for believing that Dr Stewart's statistical analysis of cancer rates at Hanford, which supported her thesis, was unsound and that the experimental data were inadequate to contradict the much larger amount of evidence taken into account by the ICRP. These criticisms have not been effectively refuted, but they were not even presented. The only statement by Dr Reissland which appeared on the programme was his answer to a question by the compere at the end of his interview asking him what would be the effect on radioactive regulations if Dr Stewart's findings were correct, to which Dr Reissland's answer was, when pressed, that *if they had been* correct it would involve a tightening up of regulations by a factor of 20. Without giving his previous explanation of the reasons showing that her conclusions were not supportable, this gave a complete misrepresentation for which no apology has ever been made.

Yours faithfully,

J. H. FREMLIN

Department of Physics, University of Birmingham, UK

Civil liberties and the nuclear critics

SIR, — Robin Grove White (20-27 December, page 774) has written a thoughtful and stimulating article on the alleged threat to civil liberties presented by an extended nuclear power programme. Unlike many (even most) critics of nuclear power, he is fair-minded enough to state that it is "conceivable" that alternative strategies would be worse. To his further credit, he takes seriously the possibility of "spying and infiltration of campaigning groups", even if he appears unsympathetic to the counter-measures available to the democracies.

However, I feel that it is unfair to intimate that there is anything peculiar to nuclear power about this latter aspect. The problem of uninvited involvement in our affairs seems to be with us anyway, as is indicated by such cases as those of Alger Hiss (according to a recent book), Philip Agee (according to a former CIA director) and Antony Blunt (according to himself). It is something which, in my opinion, the organisers of campaigns and demonstrations ought to face up to much more than they now do.

A problem to which Mr Grove White does not address himself at all is that of the tactics of some of the anti-nuclear people themselves. There have already been cases, in several countries, of vandalism and bombing. We can, I suppose, expect this behaviour to worsen if (as I expect and support) we choose to increase the contribution of nuclear power to the solution of our energy problems. At what point would these methods become an "impact . . . on civil liberties"? If kidnappings start? Or assassinations? This is by no means a rhetorical question. To put it another way, at what stage can we expect civilised and decent critics of nuclear power to abandon their position because of the activities of some of their associates?

Yours faithfully,

J.F. CRAWFORD

Klein Doettingen, Switzerland

Dioxin detection

SIR, — With regard to Dr Warren Crummett's comments (24 January, page 330) on my report of Dow Chemical's poor performance in detecting TCDD (dioxin) (8 March 1979,

page 109), I should like to make the following points.

● Dr Crummett was asked to comment on his results at the EPA meeting and he told me that they were "poor" and "worse" than normal.

● Most of Dr Crummett's criticism of my report refers to the detection of TCDD samples below 9 parts per trillion. He is of course correct to say that the collaborators concluded that 9 ppt was the lowest acceptable detection limit and I made this point in my report.

● To my mind one of the most important issues is the reliability of measurements to detect TCDD in the 9-81 ppt range. Dow did not perform as well as the other laboratories in this study and the EPA report comparing all the results from the five laboratories bears testimony to this. Dr Crummett's comments to me at the EPA hearing only confirmed what was already in the EPA document.

Yours faithfully,

ALASTAIR HAY

University of Leeds, UK.

SIR, — My comments on Dr Hay's response are as follows:

I agree with Dr Hay that I used the words "poor" and "worse than normal", referring to the entire EPA Dioxin Implementation Plan study as "poor" and the Dow results in particular as "worse than normal".

The important issues in the reliability of the measurement of TCDD are, in order of importance: false positive results, false negative results, precision and accuracy of measurements, and confirmation by a second laboratory.

False positive results are most important because agencies in the United States, as explained by EPA's Tom Holloway at the Dioxin Implementation meeting, have a license to regulate whenever a toxic chemical is detected in environmental samples by state-of-the-art analytical methodology. Dr Hay is correct, however, in stating that the reliability of measurements in the 9-81 ppt range is scientifically important. In this range Dow and Wright State University produced comparable results on the same samples. No other laboratories examined identical extracts. So Dr Hay's comment that "Dow did not perform as well as the other laboratories" is his opinion.

I trust that this matter can now be put to rest without creating a continuous debate in your journal.

Yours faithfully,

WARREN B. CRUMMETT

Dow Chemical, Michigan, US

Suspicion of totalitarian ideologies

SIR, — Vera Rich in the article "Berufsverbote continues in spite of resolutions" (6 December, page 549) mentions the political disruption of academic life during the 1930s and remarks that it is strange that the anti-Berufsverbot campaign started so late.

But it is the 1930s that have made us suspicious of any totalitarian ideology. Left and right terror is no different, and we have already had more than enough disruption by Marxists in our universities. We should not help them to occupy influential positions in government service unless we want the suicide of democracy. There is a German proverb which says "Nur die allerdümmsten Kälber wählen ihre Schlächter selber" (only foolish calves choose their own slaughterer).

Yours faithfully,

ROLF SEIFERT

Universität Hamburg, West Germany

NEWS AND VIEWS

Was there a Maunder Minimum?

from Christopher Cullen

J.A. EDDY's claim (*Science* **192**, 1189; 1976) that solar activity was at an unusually low level during the years AD 1645–1715 (the 'Maunder Minimum') has so far seemed to be supported by the existence of a gap in East Asian naked-eye sunspot records covering this period. Two Chinese researchers at the Nanking Purple Mountain Observatory, Xu Zhenao and Jiang Yaotiao, have now drawn data from previously unused sources, and claim that on this basis solar activity can be seen to have continued unabated during the entire seventeenth century (*Nanjing Daxue Xuebao (Ziran Kexue Ban)* No 2, 31; 1976).

It is now acknowledged that the records of the Chinese State Astronomical Bureau form an invaluable body of data on celestial phenomena of all kinds, covering a period of two millennia. These data have come down to us in the form of digests given in the astronomical sections of the standard histories, *cheng shih*, of successive dynasties, which have in turn formed the basis of such works as Ho's catalogue of comets and novae (*Vistas in Astronomy* **5**, 127; 1962) and the special study of supernovae by Clark and Stephenson (*The Historical Supernovae*, Oxford, 1977). The first thorough listing of sunspot observations was that of Kanda (*Proc. Imp. Acad. Japan* **9**, 293; 1933), who also included Korean and Japanese data. Similar ground was covered in China recently by the Ancient Sunspot Records Research Group (*Tianwen Xuebao* **17**, 218; 1976), who in addition searched for short and long-term periodicities in solar activity over the past 2,000 years. Clark and Stephenson (*Q. Jl R. astr. Soc.* **19**, 387; 1978) have rechecked the sources used by these studies and find them to have located all relevant data. There is indeed not one sunspot recorded for the period AD 1645–1715.

Xu and Jiang point out, however, that such a gap in the records does not prove

that no sunspots were observed by Chinese astronomers. In the first place, the lack of records can be partly explained by the fact that the *Ch'ing shih kao*, the draft history of the Ch'ing dynasty covering the period AD 1662–1911, simply omits to give a sunspot chronology similar to those in earlier histories. The Peking observatory was occupied by Allied troops in 1900 during the Boxer Rising, and some records may well have been destroyed. Further, apart from the standard histories compiled by central government there are a large number of local records, *fang chih*, published by local authorities or on the initiative of private persons. It is from the *fang chih* that Xu and Jiang have drawn new data. Nineteen different *fang chih* contribute 21 previously unknown naked-eye sunspot records from the seventeenth century, of which six fall into the period claimed by Eddy as the time of the Maunder Minimum. With those previously known, the total of seventeenth-century naked-eye sunspot records from East Asia now comes to 33 (see Table 1).

The six observations in the period AD 1645–1715 occur at a mean interval of 11½ years. In Kanda's catalogue the mean interval between records is 12½ years averaging over the period 28 BC–AD 1743, and Eddy himself expects to find 6 or 7

naked-eye sunspot observations in 70 years of normal solar activity as we know it today. Xu and Jiang thus conclude that solar activity in the latter half of the seventeenth century was essentially normal and claim that the Maunder Minimum is an artefact of insufficient data. After an analysis of all the seventeenth-century data they confirm that the period of variation in activity was then, as now, approximately 11 years, and propose revised dates for the maxima and minima given by Wolf and by Schöve (*J. geophys. Res.* **60**, 127; 1955).

Statistical analysis of the *fang chih* data on sunspots and other transient celestial phenomena must be approached with caution. In the case of the *cheng shih* standard histories we have a series of data covering successive periods of operation of the State Astronomical Bureau. We may begin with the assumption that the Bureau did its observational work with uniform efficiency and that the historians digested their data with equal thoroughness in each dynasty. The next step is to attempt to correct for the sampling error introduced by periods of political chaos or of simple incompetence in the running of the Bureau; the completeness of records of other types of phenomenon provides a useful cross-check here. The results of this process may still be open to doubt, but we can at least be sure that at any given time we are only dealing with one group of observers, and that their data are being transmitted to us through a single channel. In the case of the *fang chih*, however, the situation is much more complex. We have little or no idea who made the observations they contain or how the data came to be recorded; this is a source of randomness absent from the central government records. Further, it is quite unsafe to assume that the *fang chih* taken together present an unbiased sample of data. In comparing the frequency with which sunspots are recorded in different periods, we should remember that some periods will be more intensively covered by *fang chih* records than others. Before the required weighting can be applied to Xu and Jiang's data a considerable amount of

Table 1 Summary of all known seventeenth century naked eye sunspot records from Far Eastern sources.

No.	Year	No.	Year	No.	Year
1	1603 K	12	1621 A	23	1638 K
2	1604 K	13	1624 K	24	1639 K
3	1613 F	14	1624 K	25	1639 K
4	1616 K	15	1624 K	26	1639 F
5	1616 F	16	1625 F	27	1643 F
6	1617 F	17	1626 F	28	1647 F
7	1617 K	18	1631 F	29	1650 F
8	1618 F	19	1635 F	30	1655 F
9	1618 F	20	1637 F	31	1656 F
10	1618 K	21	1638 F	32	1665 F
11	1618 F	22	1638 F	33	1684 F

A, Taken from Ancient Sunspot Records Research Group list.

F, *fang chih* record from Xu and Jiang.

K, Kanda.

Full texts of original observations are given in Xu & Jiang.

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bibliographical research will be needed.

The dangers of sole reliance on the official records are now clear. For example, Clark and Stephenson have suggested that a phenomenon similar to the proposed Maunder Minimum may have occurred under the Ming dynasty between AD 1400 and 1600. They argue that since contemporary official records of other astronomical phenomena are relatively complete, the gap in the Ming standard history sunspot records for that period must be regarded as significant. However, as we have seen, an identical argument applied to the Ch'ing standard history would lead to the patently false conclusion that there were no sunspots at all between

AD 1662 and 1911. Given the civil and military chaos at the end of the dynasty, a section of the Ming records may simply have gone missing in the same way as under the Ch'ing. The work of Xu and Jiang has shown that such points can only be decided after a search of the literature ranging outside the confines of the official sources. As well as the large number of local histories, such a search must attempt to cover the essays and common-place books, *pi chi*, of private scholars likely to have had astronomical interests; the yield from these will be considerably smaller, but still worth the effort. Until the results of such an arduous undertaking become available, judgment must be suspended. □

The 'blueprint hypothesis' of axon guidance

from David I. Gottlieb

DURING the development of the vertebrate central nervous system axons grow over long distances and bypass vast numbers of neurones to home in on a distinct target region where they form synapses with a characteristic subset of neurones. Although it has long been appreciated that axons have some means of recognising their final destination (see *News & Views* 282, 672; 1979) it needs to be emphasised that axons also take a stereotyped route to their destination, they do not, for example, branch extensively and resorb the collaterals which fail to hit the target. How are these routes through the developing brain marked? Some recent anatomical investigations by M. Singer and his colleagues suggest that certain non-neuronal cells of the regenerating and developing central nervous system may have a crucial role.

Evidence for this view first emerged from anatomical studies of the regenerating lizard spinal cord. Egar *et al.* (*J. Morphol.* 131, 131; 1970) showed that during the early stages of regeneration the cord consists of an epithelial tube formed predominantly of a single layer of cells called ependymal cells surrounding a central canal in continuity with the original spinal canal. An unexpected and exciting finding was that the distal portions of the ependymal cells (that is, the surface away from the lumen of the central canal) were arranged to form large extracellular channels which contained only an electron lucent amorphous material. During subsequent stages of regeneration these channels became choked with axons growing along the length of the cord and

presumably originating from neurones located more rostrally. Similar ependymal channels were found in the regenerating cord of the newt (Egar & Singer *Expl. Neurol.* 37, 422; 1972).

Numerous anatomical investigations of the central nervous system during early development have revealed the presence of large, cell free spaces but these have usually been written off as fixation artefacts. However, in studies of the embryonic newt cord Singer *et al.* (*J. comp. Neurol.* 185, 1; 1979) found large channels between ependymal cells that bore a striking similarity to those of the regenerating cord. As in the regenerating cord, the empty channels became filled with growing axons as development proceeded. In both regeneration and development the anatomical impression was clear: ependymal channels seemed to guide bundles of growing axons. Singer *et al.* proposed what they call the 'blueprint hypothesis', the central tenet of which is that ependymal channels provide the important cues which guide developing axons and result in the characteristic route taken by the major pathways of the brain. Along similar lines Katz and Lasek (*J. comp. Neurol.* 183, 817; 1979) have performed experiments in which eyes were grafted onto the trunk of developing frog embryos; axons from these eyes enter the spinal cord and grow for long distances in a highly circumscribed tract near the lateral margin of the cord. Some substrate clues must channel these aberrant axons and ependymal channels seem like reasonable candidates. Evidence for cellular channels has also been found in the embryonic mouse retina (Silver & Sidman *J. comp. Neurol.* 189, 101; 1980). In this case the channels appear transiently during development and seem to be situated in a

way that would guide ganglion cell axons back into the optic stalk as they grow. Doubtless such cellular channels will be found in other regions of the embryonic central nervous system.

Do the channels actually guide growing axons in a way which can explain the characteristic paths of the major tracts in the central nervous system? One problem with this idea is that developing tracts in the embryo are relatively long (from several to tens of millimetres) while we don't really know how long the channels are. Serial reconstruction studies are needed to settle this point. Another is that a clean test of the idea such as altering the channels to see what happens to the tracts seems impracticable. Thus it may be a long time before we know whether ependymal cells carry some of the elusive molecular markers for specificity. Whatever the final outcome, ependymal cells and channels will be objects of lively experimental interest as neurobiologists try to understand how tracts find their way. □

How plants exploit animals

from Peter D. Moore

THE ecological position of most green plants, at the base of food chains, has placed them in what seems to be a rather vulnerable position. It is natural that many of their adaptations during the course of evolution have been defensive; both morphological and biochemical features have frequently been modified so that the grazer should regard them with more respect than relish. Notable exceptions exist, of course, such as the use of animals as vectors in the transport of seed and pollen. Here the plant is at pains to excite an animal's greed in order to exploit its freedom of movement, thus rendering more effective the dispersal of the species and the free exchange of genes. The ecological and evolutionary rewards of these processes have evidently justified the necessary energetic investment.

In view of the advantages gained by plants in these mutualistic associations with their mobile dependents, it would be surprising if their response to direct grazing were entirely passive. In a systems analytical approach to this question, Lee and Inman (*Ecology* 56, 1455; 1975) concluded that although grazers consume only a very small proportion of primary production (see *News & Views* 282, 443; 1979), they exert a dampening effect on the fluctuations in plant production which result from environmental oscillations. In this way they may contribute to the stability

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of the ecosystem, which could be considered a benefit to all member organisms.

Owen and Wiegert (*Oikos* 27, 488; 1976) sought for more tangible benefits to plants from the existence of a contingent of grazers in their midst. They asked the question in the forcible form "Do consumers maximize plant fitness?", and their guarded but affirmative reply was based on some detailed illustrations from nature. For example, they use the figures of Llewellyn (*J. appl. Ecol.* 9, 261; 1972) to show that a population of a million aphids inhabiting a single lime (*Tilia*) tree, 14 m in height, could release sugar as honeydew at the rate of 407 g day⁻¹. Llewellyn's overall annual estimate of sugar rain was 1 kg m⁻². This will obviously have a profound influence on soil microbial activity and Owen and Wiegert single out for attention the free-living nitrogen-fixing microbial genus *Azotobacter*. The energetic stimulation of this component of the soil flora could result in higher rates of nitrogen input to the soil and consequently the ultimate encouragement of growth in the vegetation. This assumes, of course, that *Azotobacter* will be the sole or main beneficiary from the aphidic manna, which is not necessarily so, since the ecology of the competitive interactions among soil microbes is likely to be complex, but it is nevertheless reasonable to suppose that some increase in nitrogen fixation will occur. The important question is whether the nitrogen gain to the plant is worth the carbon loss.

A still more direct example of a stimulating consumer has been provided by Boscher (*Oikos* 33, 451; 1979) in her studies of the cultivated leek, *Allium porrum*. This plant is frequently blighted by the leek moth *Acrolepiopsis assectella*, the larvae of which feed on leaves, scapes and pedicels, though not normally on the flowers, which Arnault (*C. hebdom. Acad. Sci. Paris* 280, 2487; 1975) considered to be toxic to them. Boscher found that moderate defoliation of the leek had no adverse effect on fruit production, indeed some plants produced more fruit. Severe defoliation resulted in a considerable development of vegetative bulbils in the inflorescence. This is a reproductive strategy which allows rapid expansion of the population, since the bulbils produce plants which are sexually mature in their first year whereas plants grown from seed only flower in their second year. The switch to vegetative propagation is thus controlled by the consumer organism.

There are, of course, some plants which have themselves turned predator; some fungi trap and consume detritivorous arthropods, and there are the insectivorous pitcher-plants and sundews. But a more subtle system for the exploitation of animals as an energy resource has been developed by some tropical epiphytes which have formed associations with ants. These were first described back in the

The parity of the pion

from John Edgington

TEXTBOOKS of nuclear physics are not noted for their flair and style. A rapidly developing subject, using difficult theoretical techniques to interpret and codify great quantities of experimental data, nuclear physics rarely affords us glimpses of the essential simplicity of nature, and the standard pedagogy tends to reflect this. However a result recently obtained by C. Wilkin (*J. Phys. G*, L5, 1980), though not new, is so refreshingly simple in its derivation that it should rapidly find its place in undergraduate courses. The topic is the intrinsic parity of the π -meson.

The original (and so far only) determination of the pion's parity was that made by Panofsky at Berkeley in 1951, confirmed in 1954 by Steinberger at Columbia. Drawing on an idea of Ferretti dating from 1946, the argument has been outlined in many textbooks since, notably in Bethe and the Hoffmann's semi-classic 'Mesons and Fields'. The reaction studied is $\pi^- + d \rightarrow 2n$; arguing from general conservation principles one shows that the very existence of this reaction requires the pion to have negative parity. The reasoning is unambiguous but requires knowledge of the initial quantum state of the π -d system; the argument is that the pion cascades down through its electron-like orbits until the final strong interaction occurs in the lowest ($l=0$) orbit. This assumption, though highly plausible, is not absolutely compelling. That the pion's parity is negative is not seriously in doubt, but it would be comforting to have a slightly more watertight experimental proof.

Wilkin has provided such a proof. He uses some results obtained at SIN, Switzerland, by a group led by R. Hess of Geneva University, and reported to the 8th ICOHEPANS* Conference at

Vancouver last August. Hess *et al.* have been studying the reaction $p + p \rightarrow \pi^+ + d$, using a polarised beam and target; they have measured the dependence of the cross section on the relative spin orientations of target and projectile. Their motivation was to provide further data on the spin-dependence of the nucleon-nucleon interaction, and the present result is an amusing and unexpected bonus.

Wilkin points out that, in the forward direction, there is a simple relation between two coefficients, A_{LL} and A_{TT} , both of which measure the difference in reaction probability between states whose proton spins are aligned parallel, and anti-parallel, to one another. A_{LL} refers to the case where the spins are directed parallel (or anti-parallel) to the projectile's motion, while A_{TT} refers to the case where the spins are at right angles to this. Wilkin shows that

$$A_{LL} \pm 2A_{TT} = 1$$

the positive (negative) sign being taken if the pion has positive (negative) parity. By making a small extrapolation of the data of Hess *et al.* to the forward direction, he finds $A_{LL} = -0.3$, $A_{TT} = -0.6$; these values, with their rather small uncertainties, conform very comfortably with the notion that the parity of the pion is negative. Positive parity is unambiguously ruled out. Since A_{LL} may not exceed unity, positive parity would forbid A_{TT} from being negative, but it manifestly is so. This simple, yet pleasing demonstration of the pseudoscalar nature of the pion seems to have no controversial aspects.

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*International Conference on High Energy Physics and Nuclear Structure.

eighteenth century, but have recently been examined more closely by Janzen (*Biotropica* 6, 237; 1974) and Huxley (*New Phytol.* 80, 231; 1979). The stems of these plants are swollen and become hollow as a result of tissue death early in the life of the plant. The inner surfaces of these cavities are covered with warty protrusions. The hollow stems are invariably occupied by ants, and often by a variety of other organisms. The ants deposit quantities of detritus within these cavities and it was suggested during the last century that this could provide a source of inorganic elements for the plants if they were able to absorb them.

In laboratory and field experiments on two of these ant plant species, *Myrmecodia* and *Hydnophytum*, Huxley found that, in the presence of ants or of added inorganic nutrients, the plants grew more rapidly

than without these. When ants were fed on honey water containing ³²P as phosphate and ³⁵S as sulphate and as methionine, subsequent autoradiography showed an accumulation of radioactivity around the surfaces of the warts and also in the plant vascular tissue. So the plant can absorb these elements, possibly by way of faecal material in the cavities. Rickson (*Amer. J. Bot.* 66, 87; 1979) has conducted some similar experiments in which he provided the ant plant and its resident ants with a supply of fly larvae labelled with ¹⁴C. Within 6 to 8 weeks radioactivity could be detected throughout the plants, demonstrating absorption and translocation of organic material either directly from the decaying fly larvae or indirectly through ant faeces.

Rickson proposes that the ants donate a proportion of their food to the host plant,

but this implies both that the ants carry in material in excess of the ant colony's needs and that the route of movement of ^{14}C is directly from the food material to the plant. From the data available at present, it is still possible that the ants are acting entirely out of self-interest and are consuming all food entering the cavity so that ^{14}C entering the plant is derived only from faeces. If this latter explanation is correct, then the ant loses nothing from the association since there is no undue energy investment on its part. In either event, the plant very obviously gains from the association in both its organic and

inorganic uptake. The presence of the ants must therefore represent an energy resource for the plant which supplements its photosynthetic intake. It could be regarded as a symbiotically adapted facultative saprophyte.

The coevolution of plants and animals has led to such very obvious structures as flowers and thorns and to the evolution of a variety of chemical compounds from tannins to cardiac glycosides. It should not come as any surprise, therefore, to find that in taking advantage of the grazer the apparently passive plant is less green than it's cabbage-looking. □

New ways of applying pesticides

from Graham Richards

AT the biochemical level there is much in common between medical pharmaceutical research and the development of pesticides for agriculture. The great difference lies in overcoming the problem of application which is so much more difficult when dealing with plants. Researchers in this area have likened their problem to attempting to cure the common cold by dropping aspirins from an aircraft and hoping to catch a fair percentage of the population looking skywards with their mouths open. Small wonder then that the paper which caused the greatest interest at the 1979 British Crop Protection Conference held in Brighton* was the description by R.A. Coffee (ICI Plant Protection Division, Haslemere, Surrey) of an electrodynamic method suitable for the deposition of ultra-low volume droplets of insecticides, fungicides and herbicides.

In principle, the technique is akin to the famous Millikan oil drop experiment whereby the charge of the electron was first measured, and is similar in some respects to methods used in paint spraying. The sensational qualities are that Coffee's new design is free of any moving parts and can be run on torch batteries. Above all the droplets have curved trajectories so that when spraying the front of a plant the chemical is also delivered to the back.

The system which can be hand-held uses a gravity feed to supply liquid to a high-voltage nozzle. This voltage is achieved from the torch batteries using a solid-state converter. The emergent charged liquid drops are subjected to an intensely divergent electric field which is sufficient to carry the droplets to the target which is at earth potential. This field strength is about two orders of magnitudes greater than the gravitational pull, making for very much reduced drift and wind influence.

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*British Crop Protection Conference — Pests and Diseases was held in Brighton, UK, on 19–22 November, 1979. Vol III of the Proceedings will be published in March 1980.

The almost magical quality of being able to deliver pesticides to the hidden surfaces of a plant when apparently spraying only one side arises from the curved flux lines of the electrically propelled droplets. As the

Poisson equation demands, the lines of force are orthogonal to the equipotential contours so that the spray diverges from the high voltage nozzle only to converge on the earthed target and thus to penetrate to surfaces which are not visible on a direct line of sight from the sprayer.

A nozzle flow rate of about 0.1 ml s^{-1} corresponds to a spray volume of as low as half a litre per hectare; the droplets being of about $100 \mu\text{m}$ diameter (a power of 18 mW). This ultra-low volume application has many attractions. Most obviously it should lead to a significant reduction of environmental problems associated with crop spraying. The simplicity of the device should also mean that it can be used by peasant farmers in the less sophisticated parts of the world where the introduction of chemical aids can produce as much as a 10-fold increase in land cultivation.

Finally the device is an encouragement to chemists and researchers in crop protection since if the delivery of their compounds becomes more efficient then more expensive chemicals become economically viable and one constraint on their research is at least slackened. □

Inbreeding among zoo animals

from Robert M. May

IMPROBABLY located off Wiltshire Boulevard in Los Angeles is a small park containing the Rancho la Brea tar pits. Here the bones of creatures — dire wolves, sabretooth tigers, mammoths, ground sloths — from the romantically envisioned 'Rancho la Brea fauna' may be seen, both in existential clutters of bones in the original tar pits, and in analytic exhibits. These large mammals flourished in North America until comparatively recently, when 35 of 49 genera became extinct in the short period from roughly 11,000 to around 8,000 years ago; many people believe the extinctions were caused by the arrival in North America of the comparatively advanced Clovis and Folsom hunters at that time (Martin *Nature* 212, 339; 1966).

The largest bird of those times was the now-extinct *Teratornis incredibilis*, whose wingspread of about 16 feet makes it the biggest soaring bird of prey yet to have lived. Its smaller congener, *T. merriami*, with a 12-foot wingspan and an estimated weight of 50 lbs, followed it into oblivion. The greatest bird of the Rancho la Brea fauna that survives is the California condor, with a wingspan of over 9 feet and a weight around 23 lbs; here I resolve the taxonomic pedantry of whether the Pleistocene *Gymnogyps amplus* and the present-day *G. californianus* are really two species, or the same species separated in time, by referring to both as the California condor. (Note that the three

birds, which occurred together over part of their pristine ranges, form a nice Hutchinsonian sequence, with length ratios around 1.3 and weight ratios around 2; for an irreverent discussion of these ratios, see *Nature* 270, 660; 1977.)

The California condor's chances of continued existence are, however, slim. Its Pleistocene range, west of the Rocky Mountains from British Columbia to Baja California and east of the Rockies in a great southern strip extending to Florida, has narrowed to two counties in southwestern California, in the dry, mountainous parts of the southern Coast range and the Sierra Nevada. The total population has fallen from an estimated 60 in the 1940s, to 42 in 1963, to 30 today. Some of the loss is demonstrably due to wanton shooting by idiots exercising their 'right to bear arms'. The breeding rate (typically one egg per year) is not calculated to rebound from such vicissitudes. Even this potential recruitment is not being realised, possibly because of the many disturbances associated with increasing human encroachment in the habitat, and possibly because pesticides in the food supply are weakening the birds' egg shells.

There is currently much controversy about what to do (see *NY Times*, 23 Dec. 1979, A17). Various Federal and California agencies, along with the National Audubon Society, have signed an

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agreement to commit resources to a "condor recovery plan". The proposal is to mark and fix radio transmitters to the surviving condors, hoping to gain more precise information about their autecology and about the causes of the continuing decline in population. The plan contemplates capturing 9 of the remaining 30 birds for captive breeding in zoos. Some conservation groups have attacked these proposals, on the grounds that (in the words of David Brower, founder of Friends of the Earth): "The condor deserves better than to be pushed toward extinction by enthusiasts of technological fixes. The proposed plan threatens to destroy the species in a misguided effort to save it". The observed trends suggest to me there is little hope for the California condor in the wild, no matter what is done.

If this gloomy view is correct, the condor will, at best, join an increasing band of creatures who live on only in zoos. There are many consequent problems. One class of difficulty has to do with getting the animals to breed in captivity; thus, although Andean condors have produced offspring in zoos, California condors have never yet done so. Another concern, which has received less discussion, has to do with the necessarily reduced genetic diversity associated with the small founding stocks of zoo populations. In particular, many zoo stocks will be inbred to a significant degree.

An important recent study of the effects of inbreeding in zoo populations is by Ralls, Brugger and Ballou (*Science* 206, 1101; 1979). They examine records bearing on the relation between inbreeding and juvenile mortality for 16 species of captive ungulates. The offspring were separated into two classes: "noninbred" for all young with unrelated parents; and "inbred" for all young whose parents were known to have an inbreeding coefficient greater than zero. The authors believe this coarse and somewhat unsatisfactory classification scheme is forced on them by the smallness of the sample size (the scheme includes the assumption that the founding individuals were not related, which is not always true). Of the inbred young for which explicit calculation of the inbreeding coefficient is possible, 75% had inbreeding coefficients in excess of 0.25. Within these two classes, young that survived 6 months or more were considered to have "lived", while the "died" category included all offspring surviving less than 6 months (including stillborn and those born prematurely).

The results are shown in the Table. The intuitive impression of a significantly higher juvenile mortality associated with inbreeding is confirmed at the 95% confidence level for 9 of the 16 species, and the sign of the effect is in the right direction for 15 of the 16 (kudu being the exception). In a different analysis, the authors showed that for "19 of 25 individual females, belonging to ten species, a larger

Juvenile mortality of inbred and noninbred young.

Species	Noninbred young		Inbred young	
	Lived	Died	Lived	Died
Indian elephant	11	2	2	4
Zebra	20	7	3	2
Pygmy hippopotamus	139	45	23	28
Muntjac	18	4	12	6
Eld's deer	13	4	0	7
Père David's deer	15	2	19	3
Reindeer	19	10	9	12
Giraffe	11	3	2	3
Kudu	10	4	8	3
Sitatunga	15	1	31	28
Sable	18	4	3	7
Scimitar-horned oryx	35	2	0	5
Wildebeest	6	1	29	12
Dik-dik	10	7	7	8
Dorcas gazelle	36	14	17	25
Japanese serow	52	21	27	35

percentage of young died when the female was mated to a related male than when she was mated to an unrelated male".

This work is controversial. Although the deleterious effects of inbreeding are well known in animal husbandry, many conservationists and wildlife managers have argued that "Adverse comment about the ill-effects of inbreeding is . . . often exaggerated, and provided the area is well looked after and not overstocked, there should be no harmful effects" (Whitehead, in *Threatened Deer*, IUCN Publ., Switzerland, 1978). I think Ralls, Brugger and Ballou make it clear this is wishful

thinking, and "that deleterious effects of inbreeding are widespread in captive populations of exotic animals and have remained unrecognized only because the majority of zoos have not maintained detailed records".

It must be emphasised that nobody is suggesting that the difficulties caused in inbreeding are a reason to give up attempting to breed animals and preserve species in zoos. It is simply that these difficulties are real, and that they need to be addressed constructively (by genetic management of small populations whenever practical), rather than ignored.

Nonlinear dynamics

from N. MacDonald

NONLINEAR dynamics is an explosively expanding subject, and a recent meeting* brought together more than 400 people from a great range of disciplines, from fluid dynamics to biomathematics, with a common interest in the recent advances in understanding nonlinear phenomena.

A major part of the meeting was devoted to studies, both experimental and theoretical, of the onset of turbulence. Turbulence is characterised by complex structures, on many different scales, in both space and time. However, the onset of turbulence, that is to say the first appearance of modes of motion more complex than laminar flow, is usually studied in the time domain, by analysis of the frequency spectrum of an appropriate experimental quantity. Traditionally, the transition from simple to complex frequency spectra characterising the onset of turbulence has been thought of as analogous to a series of bifurcations of the trajectories of a dynamical system (a system defined by a finite set of ordinary differential equations). The first transition is from a steady state to a closed curve with a definite frequency of transit; the second from that closed curve to motion on a torus

(quasi-periodic motion with two incommensurable frequencies), and so on. More recently the possibility has been recognised that after one or two such bifurcations there may be a sudden jump to aperiodic motion. In the experimental data presented at this meeting (for example by G. Ahlers, Bell Laboratories, Murray Hill, and J. Gollub, Haverford College) the following features have been identified:

(1) Appearance of new incommensurable frequencies $f_1, f_2, \dots$

(2) Entrainment of these frequencies so that (for example) f_1, f_2 approach values $f_0, 2f_0$.

(3) Sudden appearance of aperiodic spectra.

(4) Appearance of subharmonics $f_1/2, f_1/4, \dots$ successively.

Certain of these features were also present in the data described by C. Vidal (CNRS, Talence) on the temporal behaviour of a stirred Belousov-Zhabotinsky chemical reaction, as the flow rate through the reaction vessel is increased.

Some reservations must be expressed with regard to this way of thinking about turbulence. As emphasised by P.C. Martin (Harvard University) essential aspects of the physics may be missed if one does not consider the spatial aspects of turbulence.

*A conference on 'Nonlinear dynamics' sponsored by the New York Academy of Sciences and organised by Dr. R.H.G. Helleman, Twente University of Technology, was held in New York on 17-21 December, 1979.

Further, the appropriate theoretical framework for fluid dynamics, the Navier-Stokes equation, is defined on an infinite-dimensional phase space, that of the velocity vector field. D. Ruelle (IHES, Bures-sur-Yvette) reviewed the progress that has been made in understanding how far it is safe to apply the concepts of dynamical systems to solutions of the Navier-Stokes equations.

Within dynamical systems there are two important dichotomies; on the one hand that between dissipative systems — the category to which any presumed analogue of fluid dynamics must belong — and Hamiltonian systems, and on the other, within Hamiltonian systems, between the integrable and non-integrable cases. J. Moser (Courant Institute, New York) reviewed recent progress in understanding integrable systems. The most difficult of the long-established integrable cases, were solved by the method of separation of variables in the Hamiltonian-Jacobi equation. The recent zoo of curious integrable cases, such as the Toda lattice, has been studied in terms of the spectral theory of linear operators. It is now possible to trace a mathematical relationship between these two apparently diverse methods, and to give a systematic discussion of the connection between the various special cases.

The classical theory of integrable systems played a considerable part in the early development of quantum theory. As early as 1917, while the Bohr-Sommerfeld semi-classical method was being actively pursued, Einstein pointed out that this method could not hope to handle any non-integrable classical problem. The rise of quantum mechanics diverted attention from this, but in recent years the practical usefulness of semi-classical methods in atomic and molecular physics has led some authors to return to this problem. M.V. Berry (University of Bristol) reviewed the work which he and his associates have carried out on the quantisation of some well-known non-integrable systems, such as Arnold's cat and Sinai's billiards (see for example M.V. Berry *et al.* *Ann. Phys.* **122**, 26; 1979).

The trajectories of integrable Hamiltonian systems stay on energy surfaces of dimension lower than that of the phase space. Some or all of the trajectories of non-integrable systems fill up (in an imprecise sense) regions of the phase space (ergodic behaviour). There has been some controversy regarding the universal nature of a mechanism (Arnold diffusion) proposed for the transition from quasi-periodic to ergodic behaviour of the trajectories of a perturbed integrable Hamiltonian system. A numerical experiment bearing on this was reported by M.A. Lieberman (University of California, Berkeley and Imperial College, London). A particle is bounced between a

plane wall and a parallel rippled wall. A transition can occur between trajectories confined to a single dimple and trajectories wandering over the plane.

Another aspect of the appearance of seemingly random features in a deterministic physical model was the subject of a talk by J. Ford (Georgia Institute of Technology, Atlanta). The model is a set of particles on a line, which are alternately free and harmonically bound. For low enough frequency of the bound particle oscillations, any blow on the end particle is transmitted as a wave pulse along the line, distance travelled being proportional to time elapsed. As the frequency is raised to a point at which there is a significant incidence of multiple collisions (a free particle striking its right-hand neighbour more than once before next striking its left-hand neighbour) the nature of the transmission of information changes. The wave pulse is damped out and the disturbance propagates like heat instead of sound, with the distance travelled proportionate to the square root of the time.

Just as the Navier-Stokes equation can be illuminated by cautious extrapolation of results from dynamical system theory, so the study of complex behaviour in dynamical systems received a considerable impulse from the striking properties of discrete mappings. The degree of complexity attained by the solutions of such apparently trivial recurrence relations as

$$\alpha_{m+1} = \alpha_m(1 - \alpha_m)$$

is now familiar (see the review by May, *Nature* **261**, 459; 1976). Much attention was paid at the New York meeting (for example in the talks by O. Lanford, Berkeley, E.N. Lorenz, MIT and M.J. Feigenbaum, Los Alamos) to the remarkably precise scaling laws that apply to these solutions. In general, for the mapping

$$x_{m+1} = f(a, x_m)$$

where for fixed a the function f has a single maximum in x , there are, as a passes through values a_n , successive bifurcations from a periodic solution of order n to a periodic solution of order $n+1$. Feigenbaum has found that the limit for large n of the ratio

$$\frac{a_n - a_{n-1}}{a_{n+1} - a_n}$$

has the value 4.6642016... He conjectures (for details see *Phys. Lett.* **A74**, 375; 1979) that similar laws may hold for the subharmonic bifurcations observed in the experiments on the onset of turbulence.

This was undoubtedly a timely and stimulating meeting. The predominant impression was of a fruitful interplay between the construction of special models illustrating striking aspects of nonlinear dynamics, and the exploration of the underlying mathematical structures. □

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100 years ago

DIFFUSION OF COPPER IN THE ANIMAL KINGDOM

The fact of the normal presence of minute quantities of copper in various members of the animal kingdom has been noticed by several chemists within the past twenty-five years. Kingzett states that he has invariably found it to be a constituent of the human brain, while Odling and Dupré, and Bergeron and Hoté have determined analytically the average amount of copper present in the liver and kidneys of human beings and domestic animals. In the latter case the average percentage of copper found was about 35 millionths. Some two years since Cloez examined the blood of a deer, and found it to contain 6 millionths of copper. The most interesting instance of the occurrence of copper in the animal creation is, however, that communicated by Prof. Church to the Royal Society in 1869. At this time he was engaged in the investigation of a peculiar, soluble, red colouring matter present in the wings of the Turaco, a bird from the West Coast of Africa. A thorough study of this pigment showed it to contain 5.8 per cent of copper, and Prof. Church established for it the formula $C_{50}H_{56}O_{19}N$ Cu. Led to seek the source of this strange factor in the animal economy of the Turaco, he succeeded in detecting copper in the fruit of the *Musa sapientum*, which forms the chief article of the bird's diet.

To these few isolated cases of the normal assimilation of copper in the animal Kingdom, Dr. M. Giunti, in the last fascicule of the *Gazzetta Chimica Italiana* (vol. ix. p.541), adds a number of interesting and diversified instances.

His attention was first directed to the subject accidentally by finding over one-third of 1 per cent of copper in the guano deposits from bats occurring in certain Italian caves. This led to an analytical examination of the bat, the results of which showed that about four ten-thousandths of the weight of the ashes of this animal consist of cupric oxide. Still bent upon finding a more ultimate source for the metal, Giunti has subjected to analysis quantities of the insects which form the food of the bat, and in all cases he has found copper present in greater or less amount.

SIZING AND MILDEW IN COTTON GOODS

Sizing and Mildew in Cotton Goods. By G.E. Davis, C. Dreyfus, and P. Holland. (Manchester: Palmer and Howe).

The application of a certain kind of science to a certain kind of commerce is rapidly producing a literature of its own. It is not long since that we had occasion to notice a work which treated of the manner in which silks could be "weighted" by chemical means, and the volume now before us is the second of its kind which is concerned with the relations of chemistry and mycology to the manufacture of cotton goods.

It is hardly worth while to take up valuable space by noticing the merits or demerits of a book such as this, the object, or at least the tendency, of which is to show the manufacturer how, by the application of certain scientific facts and principles, he may seek to perpetuate a system which, we honestly think, is simply a gigantic fraud.

From *Nature* **21**, 29 Jan., 305, 298; 1880.

REVIEW ARTICLE

The mechanism of protein secretion across membranes

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Many secreted proteins are synthesised as a large precursor with an additional hydrophobic N-terminal signal sequence that is cleaved by a membrane-bound enzyme. The proteins are secreted as nascent chains. The work leading to the current models of protein secretion is reviewed and the value of bacterial systems in the study of protein transfer across membranes is stressed.

MOST cells, and perhaps all, secrete proteins across membranes—either directly into the extracellular fluid, or into vesicles or organelles. Study of the mechanism began with Palade's discovery, 25 years ago, that animal cells contain ribosomes bound to the membrane of the endoplasmic reticulum, as well as ribosomes free in the cytoplasm¹. In the next few years various secretory proteins were shown to be synthesised on the bound ribosomes, and various cytoplasmic proteins on the unbound ribosomes (reviewed in refs 2, 3). This distribution suggested that secreted proteins might cross the membrane as growing chains, rather than after completion. In support of this model, Redman and Sabatini⁴ used puromycin to induce release of incomplete chains from the ribosomes of microsomes (vesicular fragments of endoplasmic reticulum, spontaneously formed on cell lysis): these chains could then be released by a low concentration of detergent, which suggested that they had been transferred into the lumen of the vesicles.

To explain how the membrane-bound and the unbound ribosomes differed in their selection of messenger RNA, as well as in their location, a difference in structure was proposed. However, no such difference could be detected. It was therefore necessary to consider the alternative possibility that the ribosomes are all alike and bind mRNA randomly, and it is the mRNA that determines their location (as well as their product). In 1971 Blobel and Sabatini⁵ proposed that the mRNA might play this role indirectly, by coding for a special sequence near the beginning of the polypeptide chain that might be recognised by a membrane binding factor; the chain would then presumably be secreted while still growing.

Such N-terminal signal sequences were subsequently demonstrated on many secretory proteins. Nevertheless, that did not establish whether binding of the signal is followed by cotranslational secretion or whether the chain is first completed, then folds against the membrane surface and is subsequently transferred by engulfment by the membrane after release from the ribosome. In fact, as we shall see, both mechanisms can be found.

Recently cotranslational secretion has been directly demonstrated, particularly through the study of protein secretion in bacteria. Moreover the use of bacterial systems has allowed new approaches to the study of secretion. These approaches are based on the accessibility of the relevant bacterial membrane surface (in contrast to the endoplasmic reticulum of eukaryotic cells), and the isolation of bacterial mutants altered in secretion. In this review we shall emphasise these studies, briefly noting closely related work on eukaryotic cells, and on the formation of membrane proteins.

The signal sequence

In addition to its affinity for membrane, the postulated special signal sequence turned out to have the unpredicted property of

being cleaved from a precursor protein during secretion. On this basis the existence of such a sequence was discovered adventitiously, and independently of the suggestion of Blobel and Sabatini⁵, by Milstein and coworkers⁶ in 1972. Specifically, during a study of *in vitro* translation of mRNA (from myeloma cells) into an immunoglobulin, these workers observed that in the absence of homologous membrane the immunoglobulin light chain was formed not as such but as a larger precursor; moreover, the extra sequence, of MW ~ 3,000, was N-terminal. Milstein *et al.* therefore proposed that the extra sequence provided a signal for attaching the growing chain of this secretory protein to the membrane. This group further reported⁷ that during protein synthesis homologous membrane fragments can convert the precursor to the ultimate product; but after completion of the precursor the cleavage site evidently becomes inaccessible to the enzyme. Other laboratories also noted the synthesis *in vitro* of a larger precursor of an immunoglobulin chain⁸⁻¹¹.

These findings provided the first evidence that proteins destined for secretion have a special signal sequence, and that this sequence is removed after it has served its function of initiating transfer. Moreover, Schechter and coworkers^{10,12,13} showed that the signal sequence of an immunoglobulin light chain precursor has a marked preponderance of hydrophobic amino acids—a property that would fit the proposed attachment of this sequence to the membrane.

Subsequently a series of more refined studies (reviewed in ref. 14) was undertaken by Blobel and coworkers¹⁵⁻¹⁷, culminating in the determination of the complete amino acid sequence of the signal segment of a number of precursor proteins from eukaryotic cells. In some cases the membrane enzyme(s) that cleave this sequence were also demonstrated. These studies led to increased interest in the 'signal hypothesis', and to a more detailed model in which the signal sequence induces aggregation of membrane proteins to form a tunnel around the secreted chain¹⁵.

More recently, precursors with an N-terminal signal sequence have also been demonstrated¹⁸⁻²⁰, or inferred from the corresponding DNA sequence²¹, for several proteins that are secreted from bacteria. In addition, both in bacteria²²⁻²⁶ and in animal cells²⁷, precursors with cleavable signal sequences have been demonstrated for several proteins destined for incorporation into the membrane, rather than for secretion. The process of incorporation has therefore been viewed as a model for secretion (see, for example refs 26, 28). This view is supported by evidence (see below) that the two kinds of protein compete for the same sites of entry (even though their interaction with the membrane later becomes quite different).

The signal segments for various proteins have been found to vary considerably in sequence, and even in length. Representative examples are presented in Table 1; a more complete tabulation can be found in ref. 28.

Direct evidence for cotranslational secretion

Cotranslational secretion of a protein would be demonstrated directly if one could label the end of the growing chain protruding from a membrane and show that the other end was still attached to a ribosome as peptidyl-tRNA. Such experiments seemed feasible in bacteria (although not in eukaryotic cells), since the far side of the bacterial plasma membrane should be accessible to external modification (Fig. 1).

Efforts to label protruding chains were therefore undertaken in our laboratory³². In growing cells of *Escherichia coli* the polysomes were stabilised by rapid chilling plus addition of chloramphenicol; the cells were converted to spheroplasts to increase accessibility of the cytoplasmic membrane; and the spheroplasts were treated with ³⁵S-labelled acetylmethionyl methylphosphate sulphone (AMMP), which reacts with free amino groups (of protein and phospholipid) on the cell surface but does not penetrate cell membranes³³. The spheroplasts were then washed and gently disrupted by osmotic lysis; and the membrane-polysome complexes were separated, by sedimentation in a sucrose gradient or by gel filtration, from the free membrane fragments and the free polysomes.

The polysomes from the membrane-polysome complexes were freed of membrane by washing with detergent, and they were found to retain about 6% of the initial label in the complexes (2% of the total incorporation). More recently, use of ¹²⁵I-diaziodosulphanilic acid, a non-penetrating reagent that labels only tyrosine and histidine residues and not phospholipid, has increased about fourfold the proportion of cellular label that is recovered on polysomes³⁴. This label on the polysomes (from either reagent) is evidently attached via peptidyl-tRNA: it was released by low Mg²⁺; subsequent cleavage by dilute alkali decreased the average molecular weight by about that of tRNA; and the label was also released by puromycin, or by chain completion *in vitro*. Moreover, the product of chain completion, as expected, had an average molecular weight about twice that of the material released by puromycin. (This growth of the labelled molecules is particularly cogent evidence, for it excludes the possibility that the small amount of label in the purified polysomes was merely contamination.) Finally, the demonstration of cotranslational secretion was completed by identifying a periplasmic enzyme, alkaline phosphatase, among the products of completion of the extracellularly labelled growing chains³².

Using the same procedure, W. P. Smith in this laboratory has since demonstrated cotranslational secretion of several other proteins, in both Gram-positive and Gram-negative bacteria: α -amylase of *Bacillus subtilis*³⁴, the toxin of *Corynebacterium diphtheriae*³⁵, and penicillinase of *Bacillus licheniformis* (unpublished data, in collaboration with J. O. Lampen). Thus it seems that simultaneous translation and extrusion is a general (although not necessarily an exclusive) mechanism of protein secretion in bacteria.

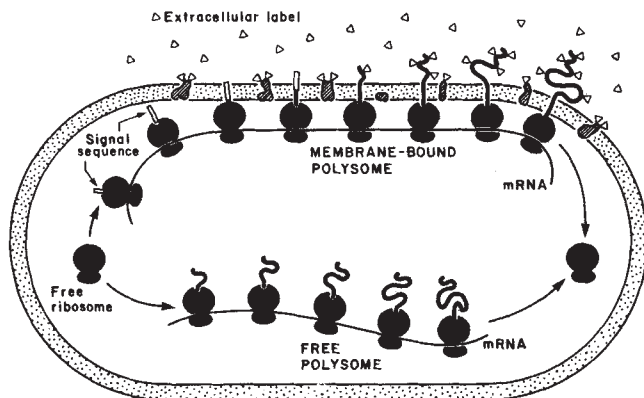


Fig. 1 Use of extracellular labelling of nascent chains to demonstrate secretion of proteins across membrane as growing chains. (Adapted from refs 32, 34.)

Recently, some intrinsic membrane proteins have also been shown to enter the membrane during chain growth, as might be expected from the demonstration²²⁻²⁶ that such proteins are formed as cleavable precursors with a hydrophobic signal sequence. Thus incomplete protein chains have been recovered from the outer membrane of pulse-labelled *E. coli* cells in steady-state growth³⁶. Moreover, in animal cells ingenious experiments have shown that during the insertion of a viral protein into the membrane of the endoplasmic reticulum, the protein can be glycosylated, on the intraluminal surface, only during the early part of chain growth and not later^{37,38}.

Significance of membrane-associated ribosomes in bacteria

The demonstration of cotranslational secretion (and incorporation into membrane) has incidentally settled the long-standing question of the functional significance of the association of ribosomes with membrane in bacteria. In thin sections of these cells, unlike eukaryotic cells, it has not been possible to distinguish membrane-bound and free polysomes by electron microscopy, because the population of ribosomes is too dense. Hence even though membrane fragments in bacterial lysates have long been observed to contain ribosomes, it has not been certain whether the attachment is functional or is due to artificial trapping. A functional role, like that observed earlier in eukaryotes, is now established.

A functional attachment was also suggested by the finding that the membrane-bound polysome fraction of *E. coli* produces a somewhat greater abundance of a secretory protein (alkaline phosphatase) than does the free fraction³⁹. Similarly, Randall and Hardy⁴⁰ prepared membrane-polysome fractions whose products were more enriched in several secreted or incorporated proteins, while containing very little of a cytoplasmic protein (elongation factor EFTu). In our laboratory W. P. Smith has achieved a more complete separation, yielding virtually exclusive synthesis of alkaline phosphatase, α -amylase, penicillinase, and diphtheria toxin on membrane-bound polysomes of various organisms, while a cytoplasmic protein (elongation factor G of *E. coli*) was made exclusively on free polysomes.

Synthesis and processing of precursors

Polysomes derived from membrane-polysome complexes have regularly yielded precursors of the secreted or membrane proteins described above, and so they appear to be a good source of precursors and intermediates in protein formation. Precursors have also been formed in purified systems directed by DNA^{18,20}, and in toluene-treated cells⁴¹. A number of these products have been shown to be more hydrophobic than the mature proteins.

The penicillinase of *Bacillus licheniformis*, formed by chain completion on membrane-derived polysomes, is a particularly interesting example, since four molecular forms can be recovered (unpublished experiments, with W. P. Smith). One of these evidently corresponds to the familiar extracellular product of MW 29,000 (29K), and another to a membrane-bound form of 33K, which has been reported to contain phospholipid^{42,43}. In addition, our 33K product could be separated on decylagarose into a highly hydrophobic and a hydrophilic fraction; and only the hydrophobic 33K fraction contained ³H-glycerol (presumably in phospholipid) when derived from cells carrying this label. Finally, an even larger form (see also ref. 20) was also recovered, and it was more hydrophobic than the secreted product, as expected of a precursor. Evidently secreted proteins in bacteria can go through successive cleavages, like the preproteins that have been recognised in eukaryotic cells as precursors of protein hormones^{44,45}. In addition, and unexpectedly, the successive cleavages of penicillinase can all occur during chain growth. Interestingly, diphtheria toxin also goes through more than one cleavage: one on leaving the bacterial cell³⁵, and another on entering the target mammalian cell⁴⁶.

Table 1 Amino acid sequence of the N-terminal signals of precursors of some secreted (S) or membrane (M) proteins

Complete sequence	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Refs	
<i>Eukaryotes</i>																											
1. Pre-immunogloblin κ -type light chain (mouse) (S)				Met	Asp	Met	Arg	Ala	Pro	Ala	Gln	Ile	Phe	Gly	Phe	Leu	Leu	Leu	Leu	Phe	Pro	Gly	Thr	Arg	Cys↓Asp	29	
2. Pre-lysozyme (hen oviduct) (S)								Met	Arg	Ser	Leu	Leu	Ile	Leu	Val	Leu	Cys	Phe	Leu	Pro	Leu	Ala	Ala	Leu	Gly↓Lys	30	
3. Pre-parathyroid hormone (bovine) (S)	Met	Met	Ser	Ala	Lys	Asp	Met	Val	Lys	Val	Met	Ile	Val	Met	Leu	Ala	Ile	Cys	Phe	Leu	Ala	Arg	Ser	Asp	Gly↓Lys	31	
4. Preglycoprotein (vesicular stomatis virus) (M)										Met	Lys	Cys	Leu	Leu	Tyr	Leu	Ala	Phe	Leu	Phe	Ile	His	Val	Asn	Cys↓Lys	27	
<i>Prokaryotes</i>																											
1. Prelipoprotein (<i>E. coli</i>) (M)						Met	Lys	Ala	Thr	Lys	Leu	Val	Leu	Gly	Ala	Val	Ile	Leu	Gly	Ser	Thr	Leu	Leu	Ala	Gly↓Cys	22	
2. Pre- β -lactamase (<i>E. coli</i>) (S)				Met	Ser	Ile	Gln	His	Phe	Arg	Val	Ala	Leu	Ile	Pro	Phe	Phe	Ala	Ala	Phe	Cys	Leu	Pro	Val	Phe	Ala↓His	21
3. Precoat protein (phage fd) (M)				Met	Lys	Lys	Ser	Leu	Val	Leu	Lys	Ala	Ser	Val	Ala	Val	Ala	Thr	Leu	Val	Pro	Met	Leu	Ser	Phe	Ala↓Ala	25

(Residues are numbered leftward from the cleavage site, ↓)

The enzymes responsible for the cleavage of signal segments are being investigated in several laboratories; so far the distinction between specific and nonspecific cleavage is hard to establish. We shall not review this topic in detail. With Gram-negative bacteria both inner²⁶ and outer¹⁸ membrane fragments have been found to carry out the reaction in various systems; in some the presence of detergent is required^{26,47,48}. Cleavage can occur with some precursors only during formation, but with others even after completion of the protein chain.

Neither the fate of the cleaved segments nor the reason for their removal is known. Perhaps retention of a signal would cause the secreted protein to reattach to the membrane, or, more generally, to have less freedom of motion and specificity of interaction.

Genetic studies

The genetic manipulation of bacteria, to yield mutants or recombinants altered in various functions, provides a powerful tool for the study of the secretory process. Thus alterations in the signal sequence may identify its essential features. In addition, mutants altered either in membrane components, the ribosomes, processing activities, energy transduction, or other, unknown factors should throw light on the mechanism of secretion.

Using an elegant technique for gene fusion^{49,50} Beckwith, Silhavy and associates⁵¹⁻⁵⁶ have isolated strains of *E. coli* in which an *N*-terminal sequence of a secreted or an incorporated protein, including its signal segment, replaces the *N*-terminus of a cytoplasmic protein, β -galactosidase (for details, see ref. 54). Those hybrid proteins that contained the *N*-terminus of the interpreted (outer membrane) protein, phage λ receptor, were found to be localised either in the cytoplasm or in the outer membrane of the cell, depending on the length of the membrane protein component⁵¹. In contrast, hybrids with the periplasmically secreted maltose-binding protein were localised in the cytoplasmic membrane, and not in the periplasm, even when they contained almost the entire maltose-binding protein (including its signal segment)⁵⁶. Thus secretion seems to depend not only on the signal sequence but also on the subsequent sequence: evidently some peptide sequences cannot pass through the secretory channel⁵⁶.

In these experiments the secretory hybrids that stick in the membrane were also found to interfere with the export of certain normal outer membrane proteins, which then accumulate in the cells in their precursor form⁵⁶. This finding provides evidence that secretory and membrane proteins initially interact with membranes in the same way, that is, enter at the same site(s); presumably a later, probably hydrophobic 'stop' signal in the non-secreted proteins causes them to leave the channel and become embedded in the lipids of the membrane⁵⁵. Such a second signal was already postulated earlier^{27,38}, on the basis of the similarity of the initiating signal sequences in the two kinds of proteins.

Other interesting classes of mutants have also been obtained. (1) In certain hybrids the function of the signal sequence has been eliminated by partial deletions, and also by various single amino acid substitutions (J. Beckwith, personal communication); the precursor then accumulates in the cytoplasm^{53,55}. Moreover, when mutations of either class are transferred genetically to the normal secreted or incorporated proteins, precursors of these proteins also accumulate in the cytoplasm. These results provide direct evidence for the essential role of the signal sequence in the initiation of secretion; in addition, the deletions define the minimal length of an effective sequence. (2) The effects of some of these mutations could be reversed by a suppressor mutation; and it is interesting that this mutation appears to map in a ribosomal protein (S. Emr and T. Silhavy, personal communication; see ref. 54). (3) Another mutation, presumably in a membrane component, greatly decreases the secretion of various periplasmic proteins, and also the incorporation of outer membrane proteins⁵⁷. (4) Finally, a point mutation in the signal sequence of the lipoprotein precursor prevents its cleavage but, nevertheless, allows its incorporation into the outer membrane (although the altered *N*-terminus can no longer be linked to the peptidoglycan)²³. It thus seems that cleavage is not required for export of a protein to the outer membrane.

Source of energy for cotranslational secretion

The hydrophobicity of the signal segment might lead to its spontaneous insertion between lipid molecules in the membrane. However, the source of energy for the extrusion of the subsequent, largely hydrophilic nascent chain is obscure. Figure 2 illustrates several possibilities.

(a) **Chain elongation on an attached ribosome:** Part of the energy expended on chain elongation on the ribosome might be used to push the chain through the membrane (Fig. 2a). This mechanism would require that the ribosome have a direct attachment to the membrane, to serve as a fulcrum. Purified microsomes from animal cells^{59,60}, and chloroplasts from plant cells⁶¹, are thought to have such an attachment, since release of the nascent chains from the ribosomes by puromycin did not release the ribosomes from the membrane, unless the salt concentration was elevated or a cytoplasmic detachment factor was provided. However, with purified membrane-polysome complexes from bacteria we found that puromycin released the ribosomes^{34,62}, even in ionic conditions (high Mg^{2+} , low K^+) that might be expected to stabilise a weak association⁶². This finding suggests that ribosomes in bacteria are attached to the membrane solely by their nascent chains. If so, the ribosomes cannot push these chains through the membrane. However, an alternative explanation for ribosome release by puromycin is not rigorously excluded: loss of a growing chain embedded in the membrane might cause a conformational change, either in the ribosome or in a membrane protein, that would disrupt a direct attachment of ribosome to membrane.

(b) Folding of the extracellular chain: After entry of the relatively hydrophobic initial segment into the lipid of the membrane a few residues of that segment, or of a later one in a loop⁶³, might somehow be forced into the aqueous phase on the far side (Fig. 2b); the spontaneous folding of the protruding chain might then progressively pull the rest of the chain after it^{34,63}. However, when the chains protruding from protoplasts were excised, by treatment with pronase, the ribosomes remained attached to the membrane⁶⁴; hence they are not held by an extracellular 'knot'. Moreover, alkaline phosphatase of *E. coli* can still be transferred across the membrane even after extensive modification (by mutation, incorporation of amino acid analogues, or premature termination yielding fragments as small as 10%: reviewed in ref. 65). Extracellular folding therefore seems an unlikely source for the energy of secretion.

(c) Embedding of the ribosome in the membrane: If the ribosome, rather than the protein chain, were embedded in the membrane (Fig. 2c) the nascent chain leaving it might already be extracellular. However, in electron micrographs membrane-bound ribosomes do not appear to be embedded; moreover, we have shown that the membrane protects part of the growing chain from proteolytic digestion⁶⁴.

These experiments on protection from digestion have shed further light on the secretory process, for they have shown that the protected length is uniform for many nascent chains traversing the membrane, and they have defined that length. In these experiments cells of *Bacillus subtilis* were pulse-labelled with a mixture of radioactive amino acids, chilled, and converted to protoplasts, and pronase was used to 'shave' the protruding chains. The membrane (plus ribosomes) protected a length of 49 residues for 50% of the chains⁶⁴. (Most of the rest were longer, and of varying length; they were presumably being folded into the membrane.) In control experiments, membrane-free ribosomes protected a uniform length of 28 amino acid residues, as noted earlier with mammalian ribosomes^{66,67}. The difference between 49 and 28 (21 residues) may be taken as the approximate size of the secreted chain surrounded by membrane. As an extended polypeptide chain this number of residues would have the same length, 75 Å, as the commonly observed thickness of bacterial membranes.

(d) An active secretory machinery in the membrane: The most plausible model for cotranslational secretion appears to be one in which an organised membrane structure surrounds the secreted chain and transports it, unidirectionally, by transducing metabolic energy (Fig. 2d). As in the active transport of small molecules, the energy could be derived from the proton-motive force or from intracellular high-energy phosphate. Such an active carrier would differ from the passive tunnel that has been postulated previously^{15,60,63}, but it shares the possibility that the 'channel' is induced, by the signal sequence, to assemble in the fluid membrane from mobile components^{15,60}.

This model is supported by several lines of evidence. Thus a uniform apparatus would be expected to enclose a uniform length of nascent chain; and the experiments with pronase described in the preceding section yielded precisely this result⁶⁴. Moreover, any machinery for actively transporting a chain, unlike a passive tunnel, would be expected to hold the chain firmly; and indeed, after elimination of the extracellular chains on protoplasts by pronase the subsequently isolated membrane-polysome complexes retain their ribosomes, even when incubated at 37 °C for 30 min⁶⁴. Finally, the postulated apparatus would presumably contain specific proteins; and in the membrane from the membrane-ribosome complexes of *B. subtilis* we have observed two major protein bands that are absent from the free membrane fraction (D. Marty, P.-C. T. and B. D. D., unpublished).

Nevertheless, the significance of the extra proteins is not certain. Two additional membrane proteins have been observed in the ribosome-bearing fraction of the endoplasmic reticulum in animal cells and have been termed 'ribophorins'^{68,69}, because they are believed to mediate direct binding of ribosomes. Although our evidence argues against such a direct attachment

in bacteria, we have noted that a conformation-dependent attachment would be difficult to exclude; and such an attachment, continuous with a passive membrane channel, would also be consistent with the lines of evidence cited in the preceding paragraph. The question of an active or a passive channel for protein secretion is thus still unsettled.

Alternative mechanisms for export and insertion

Cotranslational secretion, although widespread, is not the only mechanism for protein export. The suggested⁷⁰⁻⁷² alternative of secretion (or incorporation into membrane) after synthesis in the cytosol, has been demonstrated in both prokaryotes and eukaryotes, with or without formation of a cleavable precursor. We shall only briefly review some key observations.

In prokaryotes cytoplasmic synthesis, in precursor form, has been demonstrated for subunit A of cholera toxin⁷³ (which is later combined with subunit B and secreted). Synthesis of a cytoplasmic precursor followed by insertion of a cleaved product into the cytoplasmic membrane has been reported⁷⁴, but disputed⁷⁵, for a phage coat protein. In eukaryotes, cytoplasmic synthesis of a precursor has been observed for a subunit of the ribulose-bisphosphate carboxylase of the chloroplast interior⁷⁶⁻⁸⁰, and for several subunits of ATPase⁸¹ and of cytochrome oxidase^{82,83} of the mitochondrial inner membrane. The oxidase subunits are formed by several cleavages of a single large precursor, while other subunits of the same transmembrane protein are made within the mitochondrion^{82,83}. It is not clear why the cleavable signal segment of certain proteins fails to direct the polysome to become membrane-bound, or what role this segment plays in triggering⁸⁴ the interaction of the completed protein with the membrane. Nor is it clear why ATP is required for the incorporation of some cytoplasmically synthesised mitochondrial proteins⁸⁵.

Other proteins are secreted or incorporated in the form in which they are synthesised, that is without the need of a precursor form. In bacteria these include colicin E1 and E3⁸⁶, and several products of a sex factor⁸⁷; whether these proteins are synthesised by membrane-bound or by free polysomes is not known. In eukaryotes the best example^{88,89} is ovalbumin (in contrast to other proteins secreted by the hen oviduct^{30,39}). Ovalbumin is particularly interesting: its N-terminal region is not highly hydrophobic⁸⁸, and a hydrophobic segment in the middle of the molecule may serve as the signal. This conclusion was based on the finding that secretion of another protein (from a precursor) into vesicles *in vitro* can be competitively inhibited by this segment, either exposed in the incomplete or the denatured chain⁹¹, or isolated as a fragment from the molecule⁹².

Cytoplasmic synthesis preceding secretion seems to be incompatible with provision of energy by a membrane-attached ribosome; it would be compatible either with embedding of the folded protein in the membrane (followed, for secretory proteins, by release on the other side), or with insertion of the unfolded chain into an active machinery of transport. In addition, if ovalbumin initiates insertion at an internal sequence it would have to be drawn through the membrane as a long hairpin, or as a folded protein; this process also seems incompatible with being pushed by the ribosome through a passive channel.

Membrane morphogenesis

Eukaryotes (and some prokaryotes) have more than one kind of membrane in the same cell, thus raising the broad problem of morphogenetic specificity. Specific signal sequences may well determine the choice of different membranes by different growing proteins—a possible role that enhances interest in these sequences. A good model system with which to study this

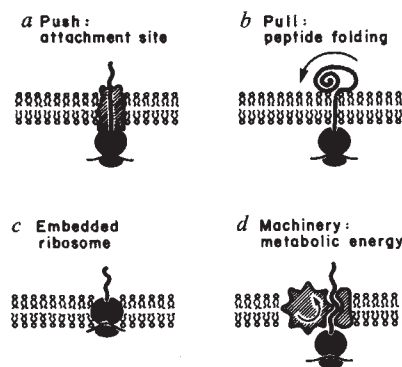


Fig. 2 Models for cotranslational chain extrusion across membrane. *a*, The energy derives from the GTP hydrolysis required for chain elongation on the ribosome; this model requires a firm ribosomal attachment to membrane and a fixed channel in the membrane. *b*, Spontaneous extracellular folding of nascent peptide pulls the chain through the membrane. *c*, Ribosomes are embedded in the membrane, releasing their nascent chains directly to the farther side. *d*, The membrane itself provides a machinery for transducing metabolic energy into unidirectional transfer of the chain. (Adapted from refs 58, 62.)

possibility may well be the formation of the inner and the outer membrane of Gram-negative bacteria. Although the proteins of the outer membrane may be secreted across the inner membrane and only then incorporated, after crossing the peptidoglycan layer, it seems more likely that they selectively enter the junctions (adhesion sites⁹³) between the two membranes and then move outwards in the two-dimensional fluid of the membrane. Thus matrix protein of the outer membrane is localised initially at adhesion sites⁹⁴, and bacteriophage λ receptor protein only at the cell septum⁹⁵.

Among the proteins in the periplasm, secretion is reported to occur only during cell division for galactose- and maltose-binding proteins^{96,97}, but throughout the cell cycle for alkaline phosphatase⁹⁶. These differences provide further evidence for diversity in the mechanisms of transfer.

Discussion

In the past 8 years, since the discovery of the *N*-terminal signal sequence of an immunoglobulin precursor, our knowledge of the mechanism of protein secretion has progressed a good deal. The process is very similar in bacteria and in eukaryotic cells, except that after peptide release, by puromycin, ribosomes remain attached to membrane in preparations from animal cells but not in those from bacteria. This difference might reflect a basic difference in mechanism, perhaps associated with a greater stability of the machinery of secretion in animal cells. On the other hand, the mechanisms might be the same, one of the two observations failing to reflect the physiological process.

The central problem of protein secretion is now the identification of the basic molecular mechanism that ensures unidirectional transfer of the growing chain. The available evidence seems to point towards a specific machinery for active transport of proteins across the membrane; but direct attachment of the ribosome, permitting it to supply the energy, is not rigorously excluded. The decision between these mechanisms must await further information, either on the nature of the contact between ribosome and membrane, or on the source of the energy for transfer of the growing chain. And among other aspects of the process, we do not know whether the signal sequence is first inserted into lipid linearly¹⁵ or as a loop^{28,63}; or whether the channel surrounding the chain exists preformed or

is induced to assemble from separate molecules in the membrane. Finally, since at least some secreted and some incorporated proteins seem to enter in the same way, the mechanism responsible for their subsequent divergence, presumably triggered by a second, later signal segment, remains to be determined. A possible model for this 'stop' signal is the anchoring of an erythrocyte membrane protein⁹⁸, and a penicillin-binding enzyme of the inner membrane of *B. stearothermophilus*⁹⁹, by a cleavable COOH-terminal segment. In the latter protein a rather hydrophobic sequence has been determined for the cleaved segment (D. J. Waxman and J. Strominger, personal communication).

It seems reasonable to expect that genetic studies will shed much light on these problems. Another approach is provided by the use of conditions that interfere with secretion by decreasing the fluidity of the membrane¹⁰⁰⁻¹⁰².

In bacteria the presence of a multi-layered rigid envelope presents a special problem for secretion of proteins into the medium: it is not known whether these proteins pass through the successive layers, or through specialised regions of the cytoplasmic membrane that are exposed to the exterior. In addition, the segregation of different proteins into the inner and the outer membrane of Gram-negative bacteria presents a problem of membrane morphogenesis analogous to that of eukaryotic cells.

Knowledge of signal sequences for secretion has recently found practical applications. For example, to increase the yield of insulin in recombinant bacteria the gene for insulin has been fused with the beginning of the gene for the *E. coli* periplasmic β -lactamase; the resulting hybrid protein is secreted, and it can then be recovered by osmotic shock and cleaved to yield the hormone¹⁰³. In addition, the bacterial mechanism of secretion can evidently recognise a mammalian signal, since bacteria containing the ovalbumin gene secrete its product^{104,105}.

The experiments described here illustrate the continuing value of bacteria as model cells, in an era that has seen a large shift away from this approach to cell biology.

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ARTICLES

Sr isotopic fractionation in Ca–Al inclusions from the Allende meteorite

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True relative Sr isotopic compositions, determined by double spiking on Ca–Al inclusions from the Allende meteorite show up to 1.5% per mass unit mass fractionation relative to the Earth and bulk chondrites. All abnormal inclusions are light-isotope enriched. A lack of isotopically heavy Sr in inclusions would place constraints on the time, place and mechanism of origin of these objects.

A FEATURE of much of recent data^{1–4} on isotopic anomalies in meteorites is that an arbitrary value for a certain isotopic ratio has been used to correct for mass fractionation in the mass spectrometric analysis. This means that the true isotopes responsible for any anomalous isotopic ratio cannot be uniquely identified, and several interpretations of mixing of nucleosynthetic components are possible^{5,6}. The double spike isotope dilution technique, however, corrects for laboratory fractionation, and allows a full comparison between the isotopic compositions of samples^{7,8}. The method leads to clear identification of the isotopes involved in nucleosynthetic anomalies, and also any natural mass fractionation processes which occurred in the sample before analysis^{9,10}.

Two spike solutions, enriched in ⁸⁷Rb, ⁸⁴Sr and ⁸⁷Sr, and differing only in Rb/Sr ratio, were both calibrated in quadrupli-

cate against NBS 987 Sr. The composition for NBS 987 of ⁸⁸Sr/⁸⁶Sr = 8.37521, ⁸⁷Sr/⁸⁶Sr = 0.71014 and ⁸⁴Sr/⁸⁶Sr = 0.056584 was assumed. The spike composition was calculated by means of the double spike mathematical solution⁸, where NBS 987 constituted the 'spike' for the determinations, and the eventual spike constituted the 'sample'. As the composition of NBS 987 is a very poor spike for this purpose, the error propagations are large; resulting spike compositions are given in Table 1. The spike allows inter-sample comparison of total isotopic composition, but cannot yield 'absolute' values for external application, because no absolute calibration standard is yet available.

Samples were handled with stainless steel tools in clean room conditions. An HF/HNO₃ bomb dissolution was used, and great care was applied to ensure total solution of the sample after this stage, first in HNO₃ and then in HCl. The total sample was then divided into aliquots in HCl solution and spiked. Completely separate sets of equipment were used for spiked and unspiked

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Table 1 Rb-Sr spike compositions

Spike	Rb/Sr	$^{85}\text{Rb}/^{87}\text{Rb}$	$^{88}\text{Sr}/^{87}\text{Sr}^*$	$^{86}\text{Sr}/^{87}\text{Sr}^*$	$^{84}\text{Sr}/^{87}\text{Sr}^*$
Rb-Sr-1	0.30005	0.008908	0.088155 ±12	0.0097440 ±14	0.95547 ±40
Rb-Sr-2	0.015265	0.008908	0.088130 ±12	0.0097365 ±14	0.95541 ±40

* Errors are two standard errors of the mean of four calibrations.

sample solutions, thus eliminating the possibility of cross-contamination. Ion-exchange separation was two-stage for Sr in PTFE columns; Rb fractions were further purified¹¹. Blanks were <0.1 ng Rb and <0.2 ng Sr; for the small samples ALL-24, 40 and 41, where the first, larger column was omitted, Rb and Sr blanks were <0.03 ng.

Mass spectrometry was performed using a VG Micromass 30, fitted with a Cary 401M amplifier since July 1978. Ion currents corresponding to the most abundant isotopes were at a level of $1.5\text{--}3 \times 10^{-11}$ A. Isotopic data were collected and reduced as blocks of 10 scans over the mass range; 25–50 such blocks constitute a run. In several cases almost all the Sr on the filament was used in the analysis, and progressive in-run mass fractionation of up to 2% per mass unit was observed; no evidence was seen in any of the runs for detectable departures from linear first-order mass fractionation (compare ref. 10 for Ca).

^{87}Sr was used as reference isotope for data collection and the double spike calculation. The equations for the double spike solution⁸ were used to propagate errors in a mathematically rigorous way, using a summation of partial differentials. Only errors in spike composition, which can be disregarded for inter-sample comparison, are excluded from the final uncertainties given in Table 2. Inclusion of this component would result in a ~30% increase in errors on isotopic ratios.

Mean values for 10 runs of NBS 987 over the period of study were $^{87}\text{Sr}/^{86}\text{Sr} = 0.70995 \pm 3$ ($2\sigma_m$), $^{84}\text{Sr}/^{86}\text{Sr} = 0.056512 \pm 16$ ($2\sigma_m$), when normalised to $^{88}\text{Sr}/^{86}\text{Sr} = 8.37521$. Baselines were always constant over the spectrum to better than 0.2%, and the difference between our values and NBS values represents a bias in amplification of peaks. Results from other laboratories (see refs 3, 12) show comparable variations, particularly in $^{84}\text{Sr}/^{86}\text{Sr}$. As our amplifier tends to overemphasise larger peaks, then the results obtained by double spike analysis of NBS 987 fall around $^{88}\text{Sr}/^{86}\text{Sr} = 8.378$, $^{84}\text{Sr}/^{86}\text{Sr} = 0.05649$, instead of the 8.375 and 0.05658 used for spike calibration. The amplifier contributes a small bias to the analyses towards higher $^{88}\text{Sr}/^{86}\text{Sr}$ and lower $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{84}\text{Sr}/^{86}\text{Sr}$.

Because this study reports small differences in Sr isotopic composition for Allende inclusions, we need to demonstrate beyond all doubt that no inter-sample bias has occurred. There are only three differences in the way in which samples have been treated: (1) ALL-24, 40 and 41 were passed through only one ion-exchange column instead of two; (2) whole meteorite and Allende matrix samples were analysed with spike solution Rb-Sr-1. Rb-Sr-2 was used for mantle nodules and Allende inclusions; (3) there was large variation of the sample Sr/spike Sr ratio, from 0.07 to 3.09 (Table 2). A value of 0.5 is considered optimum.

Table 2 shows that ALL-24, 40 and 41 are quite uncritical for the overall result of this study, and the minor difference in chemical procedure is therefore insignificant. The test analyses of NBS 987 given in Table 2 are crucial in eliminating items 2 and 3 as sources of bias. These tests were all processed using the same run data for unspiked NBS 987, so that they compare closely the results of four double spike runs. Test 1 was made using spike Rb-Sr-2, and its agreement with the other three tests, made with Rb-Sr-1, shows that there is no bias connected with which of the two spike solutions is used. Tests 2, 3 and 4 were made with Rb-Sr-1, where the sample Sr/spike Sr ratio varied from 0.19 to 1.09. As the results are identical within errors, it is concluded that no bias is associated with change in the sample Sr/spike Sr ratio. Additionally, Sr compositions close to those of NBS 987 were determined for terrestrial

analyses where the sample Sr/spike Sr ratio ranged up to 3.09 (Table 2). The small bias introduced by the amplifier is approximately constant for all analyses, in spite of variation of peak sizes between different spiked runs. Hence all the analyses reported here are closely comparable in every respect.

Terrestrial and bulk chondritic Sr

The analyses of chips of four terrestrial mantle nodules and of four bulk chondrites are presented in Table 2. Rb was not analysed for these samples. The Sr isotopic compositions plot in a tight group with the four NBS 987 analyses, and together define the Earth plus bulk chondrite field of Fig. 1.

Allende Sr

All samples were extracted from a single 450 g individual. Diameters of Ca-Al inclusions (CAI) range from 15 down to 1 mm, but none smaller than 2.5 mm were analysed. All fine-grained CAI are pink except ALL-40, which is white; ALL-24, 30, 72 and 76 are colour-zoned, with white margins. Thin sections show that of the coarse-grained CAI, ALL-4, 6, 37, 46, 56 and 70 are clearly type B (ref. 13), while ALL-7 and 68 are apparently intermediate between A and B; ALL-41 and 49 were not examined.

Plotting of the results (Table 2) on an Rb-Sr isochron diagram (not given) shows a scatter of points lying below a 4.6 Gyr line, closely similar to earlier studies^{14,15} and possibly reflecting a relatively recent disturbance^{12,14–16}. In terms of $^{84}\text{Sr}/^{86}\text{Sr}$ isotopic composition, the matrix samples plot close to the Earth/bulk chondrite field (Fig. 1), while the CAI define a continuous trend towards lower $^{88}\text{Sr}/^{86}\text{Sr}$ and higher $^{84}\text{Sr}/^{86}\text{Sr}$. This trend is completely consistent with the predicted mass fractionation path, and regression of the data gives a slope of -1.05 ± 0.18 (2σ), where MSWD = 2.3. Within error, the same line is obtained if either the coarse- or the fine-grained CAI are regressed alone. A second chip of the most abnormal sample (ALL-76, a fine-grained pink CAI) was dissolved, confirming its general position. The analysis is not an exact repeat because the inclusion was not powdered; indeed, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (Table 2) and model ages of the two chips are very different probably because of the recent isotopic disturbance. The white margin of ALL-76 has an Sr composition lying approximately half way between that of the pink interior and the Allende matrix (Fig. 1). $^{85}\text{Rb}/^{87}\text{Rb}$ ratios for three samples (Table 2) are not regarded as deviating significantly from the accepted terrestrial value of 2.593, because mass fractionation cannot be controlled or reproduced in the runs.

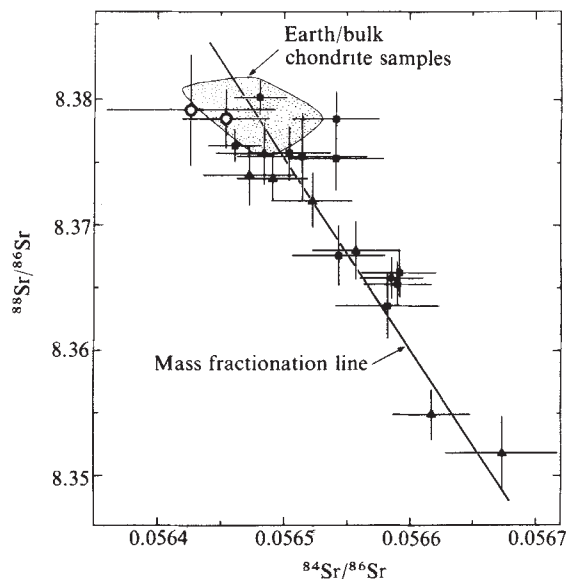


Fig. 1 $^{88}\text{Sr}/^{86}\text{Sr}$ – $^{84}\text{Sr}/^{86}\text{Sr}$ plot for the double-spike analyses reported here. Shaded area includes four NBS 987 analyses, four terrestrial mantle nodules and four bulk ordinary chondrites. All marked points, with errors at 2σ , are from the Allende meteorite, ○, Matrix; ■, coarse-grained CAI; ▲, fine-grained CAI.

Discussion

The results for terrestrial, bulk chondrite and Allende matrix samples demonstrate that the accretion regions of the Earth, ordinary chondrites and Allende matrix were essentially uniform in Sr isotopic composition.

For the Allende CAI samples, an origin for the variation of $^{88}\text{Sr}/^{86}\text{Sr}$ and $^{84}\text{Sr}/^{86}\text{Sr}$ in the recent isotopic disturbance of the meteorite^{12,14-16} can be ruled out because this would only lead to homogenisation of ratios, and could not by itself produce an apparent mass fractionation. Heavy element fission can be ruled out because ^{84}Sr and ^{86}Sr are shielded from all but direct yields. A neutron flux¹⁷ affecting all Solar System Sr except that in some CAI could explain a pattern in which Solar System ^{84}Sr became depleted and Solar System ^{88}Sr enriched relative to the CAI. However, the neutron-capture cross-sections^{5,18} show that in a neutron irradiation, burning-out of ^{84}Sr would predominate over all other effects; one-to-one correlation of ^{84}Sr and ^{88}Sr anomalies is almost certainly impossible.

The pattern of Fig. 1 can be produced by certain combinations of nucleosynthetic components. In the case of strontium, ^{84}Sr is thought to be of 100% p-process origin, ^{86}Sr and ^{87}Sr are s-process only (except for a minor contribution to ^{87}Sr from ^{87}Rb), while ^{88}Sr may be 90% s-process plus 10% r-process⁵. The compositions of the abnormal CAI relative to Solar System Sr could be explained by any of excess p plus deficient r, excess p plus smaller excess s, or deficient r plus smaller deficient s. Such nucleosynthetic explanations are unlikely for two reasons. First, in such a model, closely correlated effects are needed in partly independent components, to produce the slope of -1 in Fig. 1. Apart from this *a priori* improbability, Papanastassiou *et al.*³ have discovered, in two CAI, a nucleosynthetic anomaly in Sr

with quite different characteristics. The Sr from these two CAI cannot be plotted in Fig. 1, because of the necessity for normalisation in the runs³, but the points must lie on mass fractionation lines to the left of that in Fig. 1. Perhaps there are anomalies of nucleosynthetic origin showing good correlation of $^{88}\text{Sr}/^{86}\text{Sr}$ and $^{84}\text{Sr}/^{86}\text{Sr}$ effects in many inclusions (those of this study), and an additional, uncorrelated component present in only a few (EK1-4-1 and C1), but this seems highly unlikely.

The excellent fit of the data points to a mass fractionation line suggests strongly that mass fractionation is the physical explanation for the variation. Accepting this, the striking feature is that all the samples are either normal or enriched in light isotopes. In contrast, Si, Mg and O from CAI show either heavy-isotope enrichment, or both positive and negative δ -values¹⁹⁻²³. Although another Allende stone might show heavy-Sr enrichment in its CAI, the observed consistent light-Sr enrichment requires explanation. No clues are derived from other parameters, because the position of points on Fig. 1 is uncorrelated with inclusion type, inclusion size, major mineralogy, Sr content and Rb content. The fractionation has affected both coarse- and fine-grained CAI.

It seems likely that these CAI formed from a vapour which was isotopically lighter than normal Solar System Sr, because mass fractionation effects always lead to formation of an isotopically heavy solid and an isotopically light vapour²⁴. If the rate-determining and fractionating step in the Sr accretion process was diffusion of Sr into pre-existing solid Ca-Al objects, then light isotopes might diffuse faster than heavy isotopes, and so produce isotopically light cores plus isotopically heavy rims from normal vapour. If the outer zones were later removed by volatilisation, then overall isotopically light CAI would result,

Table 2 Sr double-spike and Rb data

Sample	mg*	sa/sp†	Rb p.p.m.	Sr p.p.m.	$^{87}\text{Rb}/^{86}\text{Sr}‡$	$^{85}\text{Rb}/^{87}\text{Rb}$	$^{88}\text{Sr}/^{86}\text{Sr}‡$	$^{87}\text{Sr}/^{86}\text{Sr}‡$	$^{84}\text{Sr}/^{86}\text{Sr}‡$
<i>NBS 987 Sr</i>									
Test 1	—	0.68	—	—	—	—	8.3792 ± 29	0.71007 ± 17	0.056491 ± 43
Test 2	—	1.09	—	—	—	—	8.3776 ± 23	0.71000 ± 14	0.056502 ± 35
Test 3	—	0.61	—	—	—	—	8.3775 ± 23	0.70999 ± 14	0.056503 ± 34
Test 4	—	0.19	—	—	—	—	8.3798 ± 24	0.71009 ± 14	0.056487 ± 36
<i>Terrestrial mantle nodules§</i>									
AJE 62	752	0.51	—	9.667	—	—	8.3806 ± 34	0.70353 ± 20	0.056467 ± 51
BD 2344	756	3.09	—	42.146	—	—	8.3800 ± 29	0.70490 ± 17	0.056491 ± 44
BD 2384	497	1.60	—	33.491	—	—	8.3813 ± 60	0.70557 ± 36	0.056469 ± 91
JJG 846	642	2.45	—	45.049	—	—	8.3771 ± 20	0.70470 ± 12	0.056479 ± 30
<i>Bulk ordinary chondrites </i>									
Parnallee LL3	702	1.02	—	10.989	—	—	8.3812 ± 37	0.76247 ± 24	0.056465 ± 57
Richardton H5	557	0.46	—	9.804	—	—	8.3800 ± 39	0.75337 ± 25	0.056462 ± 58
Barwell L5	995	0.58	—	7.527	—	—	8.3804 ± 21	0.74529 ± 13	0.056435 ± 32
Bruderheim L6	856	0.83	—	9.346	—	—	8.3788 ± 24	0.74667 ± 15	0.056516 ± 36
<i>Allende C3V matrix</i>									
Anal. 1	813	0.89	0.921	13.428	0.1986 ± 2	—	8.3792 ± 44	0.71209 ± 27	0.056426 ± 67
Anal. 2	516	0.75	0.870	13.247	0.1901 ± 3	—	8.3785 ± 23	0.71126 ± 14	0.056454 ± 35
<i>Allende coarse-grained Ca-Al inclusions</i>									
ALL-4	98	0.59	2.131	111.75	0.05513 ± 8	—	8.3755 ± 34	0.70174 ± 20	0.056514 ± 51
ALL-6	96	0.56	0.953	142.97	0.01925 ± 2	—	8.3636 ± 27	0.69893 ± 16	0.056582 ± 41
ALL-7	33	0.60	0.646	161.72	0.01153 ± 4	—	8.3662 ± 19	0.69878 ± 11	0.056590 ± 30
ALL-37	72	1.10	1.285	240.57	0.01544 ± 2	—	8.3754 ± 25	0.69934 ± 15	0.056541 ± 38
ALL-41	17	0.43	2.016	178.59	0.0326 ± 2	—	8.3758 ± 21	0.70075 ± 13	0.056504 ± 33
ALL-46	32	0.62	4.309	124.20	0.1003 ± 5	—	8.3676 ± 24	0.70456 ± 14	0.056543 ± 37
ALL-49	49	0.41	3.057	105.61	0.0838 ± 1	—	8.3802 ± 14	0.70352 ± 8	0.056481 ± 21
ALL-56	84	0.58	0.424	143.60	0.00852 ± 1	—	8.3653 ± 18	0.69862 ± 10	0.056589 ± 27
ALL-68	78	0.81	1.045	170.87	0.01768 ± 4	—	8.3764 ± 13	0.69947 ± 8	0.056461 ± 21
ALL-70	242	1.21	0.410	158.84	0.00746 ± 1	2.613	8.3785 ± 23	0.69898 ± 14	0.056541 ± 35
<i>Allende fine-grained Ca-Al inclusions</i>									
ALL-5	161	0.13	15.45	22.219	2.028 ± 7	—	8.3738 ± 18	0.79511 ± 12	0.056491 ± 28
ALL-24	15	0.10	8.438	49.675	0.492 ± 2	—	8.3680 ± 23	0.72999 ± 14	0.056557 ± 35
ALL-30	20	0.08	8.581	44.424	0.560 ± 2	—	8.3720 ± 21	0.73449 ± 13	0.056522 ± 32
ALL-40	20	0.12	6.057	31.156	0.564 ± 1	—	8.3740 ± 24	0.73318 ± 15	0.056472 ± 36
ALL-72	53	0.07	4.241	20.641	0.596 ± 1	—	8.3758 ± 25	0.73586 ± 15	0.056484 ± 38
ALL-76 margin	29	0.21	5.644	57.979	0.2816 ± 4	2.599	8.3660 ± 16	0.71503 ± 10	0.056589 ± 25
ALL-76 core	103	0.12	6.587	30.287	0.629 ± 1	—	8.3549 ± 20	0.72362 ± 13	0.056617 ± 31
ALL-76 core	28	0.07	6.245	32.066	0.5635 ± 7	2.613	8.3518 ± 29	0.73368 ± 18	0.056673 ± 45

* Weight of sample dissolved in mg.

† The atomic ratio of sample Sr to spike Sr in the spiked analysis.

‡ Errors are 2 s.e.m.

§ Mantle nodules from South African kimberlite pipes: AJE 62, Mothae; BD 2344 and 2384, Bultfontein; JJG 846, Jagersfontein.

|| Sources of meteorites: Parnallee, British Museum (Natural History), off BM34792; Richardton, Arizona State University, 100 h ASU; Barwell, British Museum (Natural History), off BM1966.59; Bruderheim, University of Alberta, B.93 UA; Allende, Smithsonian Institution.

but I feel that the generation of mass fractionation by diffusion seems unlikely. Vaporisation events, thought by many to be an important part of CAI history^{25,26}, are for the purposes of isotopic mass effects the reverse of condensation, and are thus also incapable of leaving an isotopically light solid from a normal starting material.

If the CAI formed from a vapour with isotopically light Sr, then they cannot have formed from the bulk of the solar nebula, which had overall 'normal' Sr, as shown here. They could have formed early in some restricted part of an inhomogeneous nebula^{24,27}, which had attained a light-isotope enriched Sr composition by: (1) prior condensation of materials which are isotopically heavy in Sr. These would have to be unsampled, and at least as refractory as CAI; or (2) vapour transport processes involving a kinetic isotope effect, such that one region of the vapour became enriched in light isotopes.

If the CAI did form in some restricted, fractionated region of the solar nebula, then the fact that the white outer part of the fine-grained CAI ALL-76 is closer to 'normal' Sr than the pink core may either indicate the beginning of equilibration of this anomalous region with the whole, or of the movement of that CAI as a solid object into a more normal region of the nebula. Alternatively, the white zone could merely represent equili-

bration of CAI with matrix during the isotopic disturbance demonstrated from ⁸⁷Sr/⁸⁶Sr data.

An entirely separate possibility is that the CAI are in part presolar objects^{25,28}, so that the Sr compositions are not *a priori* expected to be related to the remainder of the Solar System. Gas-dust fractionation in the presolar interstellar medium has been proposed to account for mass fractionation effects in CAI²⁹. In this scheme, the refractory Ca-Al-rich dust and grains, which are isotopically heavy, become separated from the vapour, which is isotopically light, resulting in mass separation of the elements. For the Sr data, this scheme encounters the difficulty that the Ca-Al materials seem to be all isotopically light, inconsistent with their proposed role as isotopically heavy interstellar grains and dust. Note, however, that true type A CAI have not been sampled in this study. Although the bulk Sr of the environment where the sampled CAI formed could have been isotopically light (⁸⁸Sr/⁸⁶Sr < 8.35), it would then be remarkable that this presolar bulk Sr composition was related to the Solar System Sr composition along a mass fractionation line.

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Intrusion of basaltic sills into highly porous sediments, and resulting hydrothermal activity

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Sill intrusions into highly porous sediments in the Guaymas Basin, Gulf of California, lead to low-grade metamorphism, thermal alteration and migration of organic compounds, marked changes in interstitial water chemistry, and large-scale expulsion of heated pore fluids. The latter process creates space for the intruding magma and initiates a hydrothermal system, which can explain the observed hydrothermal deposits around fault scarps on the basin floor.

STUDIES of the genesis of several land-based ore deposits show that their formation is due to the intrusion of basaltic magma into deep water marine sediments^{1,2}. Many geologists exploring such ore bodies believe that the sources of ore-forming elements are the sediments adjacent to the hydrothermal deposits^{3,4}. Due to subsequent faulting, folding and metamorphism, however,

the mechanisms of magmatic intrusions into soft sediments and their possible effects on the formation of hydrothermal deposits are not yet fully understood.

Hydrothermal deposits associated with mid-oceanic spreading centres, such as the Galapagos Rift hydrothermal system⁵⁻⁷, occur generally in conditions where sedimentation rates are

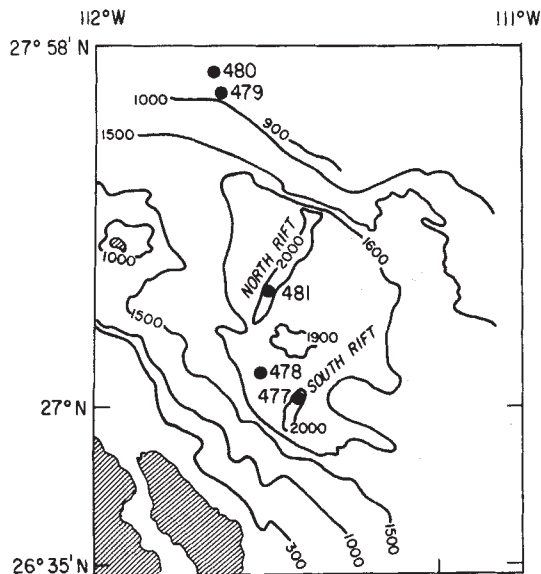


Fig. 1 Map of Guaymas Basin, depth contours in metres.

relatively slow and where magma extrudes onto the sea floor. There is strong evidence that seawater circulating through the basalts of Layer II is the convecting fluid in most mid-ocean ridge systems⁵⁻⁸. Hydrothermal waters are occasionally trapped in brine pools to form ore deposits, as has been discovered in the Red Sea⁹.

At a time when studies of marine hydrothermal systems at ocean ridge crests are gaining momentum by the discovery of hydrothermal mounds and discharge vents⁷, Leg 64 of the Deep Sea Drilling Project (DSDP) has drilled a hydrothermal system in the Guaymas Basin of the Gulf of California in a very different setting. In the Gulf of California, which represents a newly evolving ocean basin characterised by high sediment accumulation rates, oceanic basement is formed in a manner different from the more normally observed ophiolite, pillow basalt sequence of the later stages of ocean evolution.

Observations

The Guaymas Basin was formed during approximately the past 3.5 Myr by ocean floor spreading¹⁰. Two rift depressions (Fig. 1) lie in the otherwise flat floor of the basin, offset about 20 km by a transform fault. Both the north-east and south-west margins of the basin are the scarps of longer transform faults which connect to spreading axes in the next basins to the north and south. The entire basin and both rifts have been well explored by seismic and heat-flow measurements¹¹⁻¹³. Three drilling sites were occupied in the basin during Leg 64—Sites 477, 478 and 481 (Fig. 1).

All three sites drilled thick sections of turbidites, composed of muddy diatomaceous oozes and plagioclase-rich terrigenous sands to muds¹⁴. Accumulation rates exceed 1,200 m Myr⁻¹ on the basin floor and may be higher in the rift depressions. In four holes (477, 477A, 478 and 481A) basaltic to coarse-grained doleritic sills were encountered—several at shallow burial depths¹⁴ (Fig. 2).

Site 477 in the southern rift consisted of two holes, offset by only 165 m. In Hole 477 the sill consists of altered dolerite, and in Hole 477A a less altered dolerite sill was encountered at shallower depths. Conductive heat flow was estimated from shipboard measurements to be at least 20 heat-flux units (HFU = 10⁻⁶ cal cm⁻² s⁻¹), similar to that measured in the immediate vicinity by Lawver *et al.*¹³. Temperatures increase gradually with depth below the sills, thus indicating the presence of a major heat source below the drilled section of this site. Substantial increases with depth occur in dissolved chloride, lithium and manganese, especially below the sills (Fig. 3).

Dolomite, anhydrite and pyrite occur in fine-grained, clay-rich sediments below the upper sills. Below 180 m the mineral paragenesis includes epidotes (4–10% Fe), quartz, albite and chlorite. These hydrothermal minerals indicate temperatures up to 300°C (ref. 15). Carbon/nitrogen ratios of organic matter increase rapidly with depth below 150 m and the organic matter is devoid of nitrogen below 200 m. The expulsion of all organic nitrogen also indicates very high temperatures.

Site 478 is located outside the present rift zones and therefore its sediments and basaltic intrusions are probably older. Heat flow is estimated at 3.65 HFU. Only minor increases in dissolved chloride and lithium occur, and dissolved manganese values are near the detection limits (<5 µM). Various changes have occurred in the sediments around the sills, including the remobilisation of silica, the formation of dolomite, and the recrystallisation of clay minerals. No higher temperature minerals have been observed.

Site 481 in the northern rift is characterised by a heat flow of about 4 HFU. Dissolved chloride and lithium concentrations (Fig. 3) clearly indicate the influence of the uppermost sill complex. No dissolved manganese was detected (<5 µM). Hydrocarbon (C₂–C₅) gas data show a thermal component above and below the sills, with a series of three maxima above the sill. These observations are similar to those in DSDP Site 368^{16,17}. Carbon/nitrogen ratios of organic matter in Sites 478 and 481 do not change dramatically near the sills, indicating that sediments in the vicinity of the sills were not heated excessively. Oxygen isotopic analysis of recrystallised calcite, at a distance of about 1 m above the sill of Hole 481A, indicates a temperature of formation of ~150°C.

Hydrothermal system

The following observations in the sediments above the doleritic intrusions are evidence of the sill-like nature of these intrusions: (1) decreases in porosities (Fig. 2); (2) disappearance of diatoms;

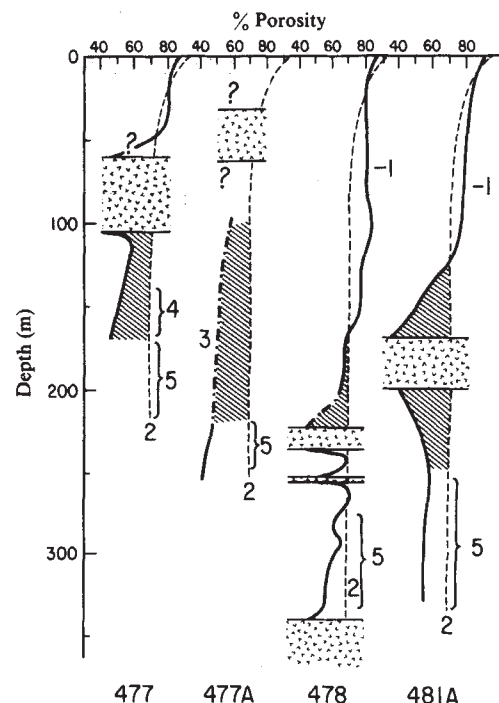


Fig. 2 Sill-induced reduction of porosity (hatched areas) in Guaymas Basin drill sites. 1, Shipboard porosity measurements; 2, shipboard porosity Site 479 (reference line); 3, porosity curve Hole 477A, deduced from density log; 4, porosity reduction due to deeper intrusions? 5, sections not considered for calculation of water loss (Table 1), though some effect may be caused by compaction due to upper sill load (in Site 478, definitely affected by lower sill).

Table 1 Reduction of porosity and water loss by intrusion of the uppermost sill (compare Fig. 2; for symbols and formulae, see text)

Hole	Thickness of sediment sections influenced by uppermost sill h_2 (m)	Loss of water (as height of water column) h_p (m)		Thickness of uppermost sill (m)
		From single sections	Total	
477	Above sill	7.3	~44	45
	Below sill	3.0		
	69*	33.4		
477A	Above sill	?	At least 75	30
	Below sill	?		
	120†	75.6		
478	Above sill	12.3	~14§	17
	Below sill	1.5		
481	Above sill	24.9	~53	30
	Below sill	28.6		

* Part of the porosity decrease of this section may be the result of a deeper hot intrusion. On the other hand, the sediments below those that were drilled are also affected by the load of the uppermost sill.

† Porosity deduced from density log. The cored section from 220 to 260 m with $n_2 \sim 45\%$ may be influenced by a deeper sill and is therefore not considered.

‡ Including thin sill at ~250 m depth. It is assumed that this sill has intruded simultaneously with the uppermost sill.

§ Minimum value, since real porosity reduction was probably larger than indicated by reference line (2) in Fig. 2.

(3) dolomite formation and calcite recrystallisation at elevated temperatures; (4) clay mineral recrystallisation and (5) organic matter decomposition.

The sharp decreases in porosity above and below the sill intrusions are particularly interesting. These decreases are clearly related to the effects of the intruding hot magmas, which cause the migration of pore waters away from the sills. The upper limits of the zone of reduced porosity presumably define the minimum thickness of sediment above the sills at the time of sill intrusion.

On intrusion of the sills, temperatures at the sill-sediment contact may rise to as high as 400 °C (the critical point for a pure 3.5% sodium chloride solution is 304 bar and 408 °C). In ridge-crest geothermal systems estimated temperatures at which seawater rock interactions occur are at least 300 °C⁷. Thus the pore waters in the immediate vicinity of the sills will rise to the boiling point. Most of the heat contained in the sill between 1,200 and 400 °C will be removed by the steam. Rough calculations indicate that for a sill of 30 m thickness, temperatures at the outer rims of the sill will drop to below the boiling point within a period of five years. From then on, heat will be lost from the sill by conduction through the sediments. Cooling of such a sill to 15 °C will be accomplished in ~50,000 yr. At sedimentary contacts in exposures on land, where original porosities may have been much lower, heating of material surrounding the intruding sills can be considerable, as evidenced by coal seams turned to cinder coal¹⁹, fusion of sediments²⁰, and clastic dykes of liquified material intruded in the sills from the underlying sediments²¹.

From the reduction in porosity in the contact zones, the amount of expelled pore water can be calculated in terms of the reduction in sediment thickness; this requires an estimate of the porosities before the occurrence of the sill intrusion. For Holes 477, 477A, 478 and 481A it has been assumed that the original porosities were similar to those in Site 479 (at the northern end of the Guaymas Basin, Fig. 1) at corresponding depths. These sediments have not been affected by intruding magma, and their composition resembles that in the basin sites. If there is a difference, the porosities in the basin sites were higher than in Site 479 because of their more turbiditic character and their faster sedimentation rates. Therefore, our calculations represent minimum values for the water losses.

The height h_p of expelled pore water can be found using the formula

$$h_p = h_1 - h_2 = h_2 \left(\frac{100 - n_2}{100 - n_1} \right) - 1$$

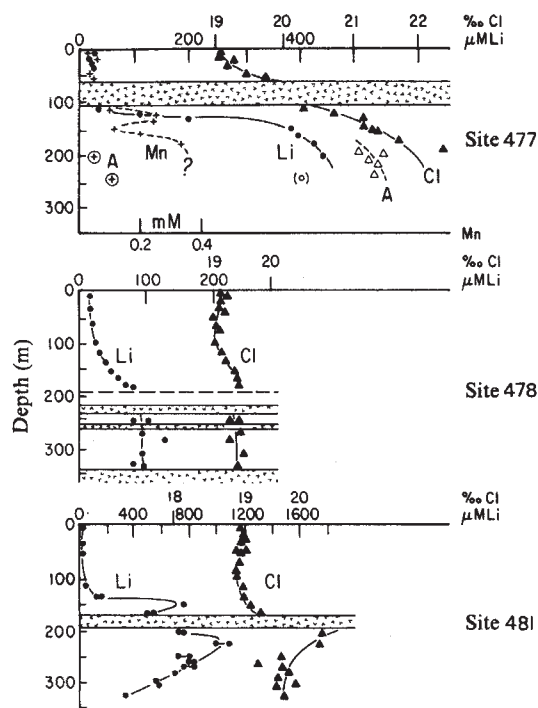
where h_1 , n_1 are the original height and porosity of a certain sediment section; and h_2 , n_2 are the present (reduced) height, porosity of same sediment section.

It follows from the above formula that h_p is not a linear function of the reduction in porosity. If the porosity is reduced from 80 to 70%, $h_p = 0.5 h_2$, that is, if $h_2 = 10$ m (present height of sediment section), 5 m of pore water has been lost and the original height of the sediment column was 15 m. If a decrease from 60 to 50% in porosity occurs, h_p is only 0.2 h_2 , and so on. Using an integrative model, the water loss from the contact zones above and below the uppermost, presumably youngest, sill was calculated (Table 1).

From the results of these calculations two principal conclusions can be drawn:

(1) At all sites the total water loss from the sediments caused by the uppermost sill is of the same order of magnitude (in height of sediment column) as the thickness of the sill. This suggests that the water removal from the neighbouring sediments creates space for the intruding magma and that a sill intrusion into highly porous sediments does not necessarily cause a large change in relief at the sea floor.

(2) If a sill of 30–40 m thickness can expel, on average, a water column of ~40 m (10 m above and 30 m below the sill), and if it occupies (perhaps together with neighbouring sills) an area of 1 km², ~40 × 10⁶ m³ of pore fluid are forced to the sea floor. This water migrates through somewhat tilted beds of relatively good permeability, for example, the sandy base of turbidites, until it reaches one of the many faults known from seismic records. These act as conduits leading the heated waters upwards (Fig. 5). At some later stage, some of the turbidites may become lithified and form cap rocks. But fissures within these layers or in the cooling sill create sufficient permeability for water to escape laterally. The sill also provides additional overburden pressure on the underlying sediments, causing compaction and thus further water expulsion. Sediments precompact

**Fig. 3** Distribution of interstitial water lithium, chloride and manganese in Sites 477, 478 and 481.

by the intrusion of older, deeper sills probably are only slightly affected by the younger intrusions.

Preliminary data on the inorganic chemistry of the pore fluids of these sites indicate that with the expulsion of pore waters and the incomplete reaction of magnesium, substantial quantities of magnesium, silica, calcium and perhaps manganese, iron and phosphate, can be brought to the sea floor. This in turn can lead to the formation of hydrothermal deposits similar to those observed in the northern trough (talc, pyrrhotite, copper and zinc sulphide, manganese oxides)²². Preliminary calculations suggest that the emplacement of a 40 m thick sill could lead to the expulsion via the pore fluids of Ca, Mg and SiO₂ of the order of magnitude of 1–50 × 10⁶ kg and ~0.01–0.1 × 10⁶ kg of phosphate per km² of sill.

Geochemistry of hydrothermal sediments

Hydrothermal alteration of basalts at oceanic spreading centres leads to large increases in ³He, Li, K and Ca in the hydrothermal fluids^{7,23,24}. The data presented in Fig. 3 also indicate large increases in dissolved lithium. Although the source for this lithium may partly be due to hydrothermal alteration of the sediments, the enhanced ³He levels observed in the bottom waters of the Guaymas Basin²⁵ indicate some magmatic contribution to the hydrothermal waters. Reversals in Li above and below the sill complex are probably related to the uptake of lithium into the sills from the pore fluids during present low-temperature alteration processes. Increases in dissolved chloride can be explained in terms of steam formation as well as by hydration of the sills.

The hydrothermal system of Site 477 is of particular interest. In this site, hydrothermal activity is associated with a deeper-seated heat source and not with the upper sills, which apparently have already cooled down considerably. Hydrothermal activity has probably been a long continuous process, leading to noticeable amounts of hydrothermal minerals, to increased metasomatism, and to the formation of high temperature phases such as epidote, albite, and chlorite. In Sites 478 and 481A, during and shortly after the intrusion of the igneous sills, heat flow must have been higher than at present. Most of these sills are considerably thinner than those observed in Holes 477 and 477A, so that cooling of these sills must have occurred at relatively rapid rates. This may explain why, in these circumstances, only moderate-temperature authigenic minerals (100–200 °C) have been detected, for example, dolomite, recrystallised calcite, recrystallised clay minerals. Also, only minor thermal decomposition of organic carbon has occurred in these sites. The three maxima in (C₃–C₅) hydrocarbon concentrations above the sill complex of Site 481 can be explained in terms of probable upward distillation (in three pulses) of C₃–C₅ hydrocarbons due to successive sill emplacements.

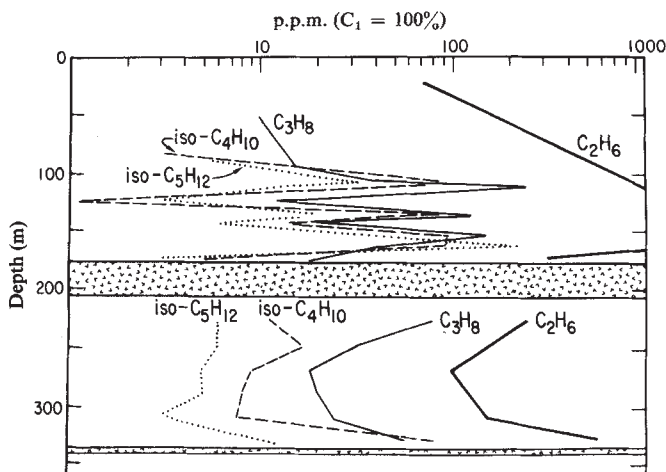


Fig. 4 Distribution of C₂–C₅ hydrocarbons, Site 481.

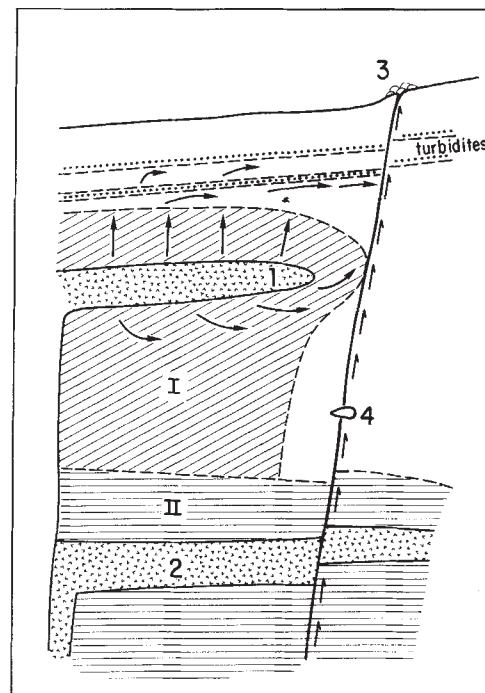


Fig. 5 Model of water expulsion due to porosity reduction as a result of intruding sills. 1, Uppermost sill; 2, older lower sill; 3, hydrothermal deposits around fault scarp; 4, Older, buried hydrothermal deposits. Area I, porosities affected by most recent sill intrusions; area II, porosities affected by older sill intrusions.

Conclusions

Drill sites in the Guaymas Basin in the Gulf of California demonstrate that during the initial stage of opening of some ocean basins, basaltic magma forming new oceanic basement intrudes as sills into rapidly deposited soft sediments in deep rift basins, thus creating a basement quite different from the normal ophiolite model concept¹⁰. Interaction between the hot sills and the surrounding sediments results in large changes in pore fluid composition (increases in dissolved lithium, chloride, manganese), in alteration of organic matter, in the formation of hydrothermal minerals, and in the large reduction in porosities in the sediment–sill contact zones. Large decreases in porosity imply substantial migration of heated pore fluids out of the sediments. This type of hydrothermal system is unlike those previously described on continents or at oceanic spreading centres. On the continent, hydrothermal systems are fed by recharge of meteoric water from neighbouring highlands, for example, the Salton Trough hydrothermal field²⁶. At oceanic spreading centres without rapid accumulation of sediment, seawater circulates convectively through the basalts of Layer II. Our suggested mechanism does not require recharge of seawater into the sediments.

The dissolved constituents of the upward-migrating pore fluids deliver sufficient material for the precipitation of large amounts of magnesium silicates (as talc) and smaller quantities of iron and manganese compounds as oxides or sulphides, as well as minor amounts of phosphates and trace metals.

While the sills cool, the expulsion of pore waters slows down and finally ceases. The hydrothermal deposits formed at the sea floor are rapidly buried by younger sediments. New hydrothermal activity starts with the intrusion of younger sills into the overlying sections of unaltered sediments. Younger sills tend to develop on top of older ones because the magma can penetrate more easily into soft sediments than into sediments compacted by older sills.

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Coding potential and regulatory signals of the polyoma virus genome

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The complete DNA sequence of the A2 strain of polyoma virus has been determined. It consists of 5,292 base pairs. The sequence is analysed in terms of its coding potential and sites of possible functional significance or structural interest. The polyoma virus genome is compared with those of related tumour viruses, simian virus 40 and BK virus.

POLYOMA virus is a small DNA tumour virus which was first isolated by Gross¹. Polyoma virus grows in culture in a variety of mouse cell lines. It transforms other rodent cells (such as hamster or rat) in culture and can produce tumours in rodents. It is now considered reasonably certain that it codes for six proteins. These are the three 'early' proteins which have been designated as small, middle and large T antigens, and the three 'late' capsid proteins designated as (virus proteins) VP1, VP2 and VP3. Whether these are the only proteins encoded within the viral DNA and whether all the proteins are entirely virus coded is not known. Only a few distinct viral mRNA species have been identified, and, as yet, no early mRNA has been directly correlated with an early protein. The great interest in the 'early region', that is, the region transcribed before DNA replication occurs, follows from the fact that it is this region, or part of it, which seems to be responsible for the transformation *in vitro* of cells or for tumour formation *in vivo* (for general reviews see refs 2, 3).

Study of viral functions has been hampered by the fact that they are difficult to distinguish from those of the host cell. For example, infection with polyoma virus results not only in viral DNA replication, but also in the stimulation of cellular DNA replication. Thus, at the same time that viral functions begin to be expressed, many cellular functions are probably also switched on. There is also the problem, for instance, that virus proteins and messenger RNAs represent only a small proportion of the total proteins and mRNAs in a cell.

The DNA, on the other hand, is in relative terms, easier to study. The genome exists as a small, double-stranded, closed-circular molecule, the physical properties of which allow it to be isolated free from cellular DNA and characterised by the techniques of modern molecular biology. In sequencing polyoma virus DNA, we hoped to circumvent some of the problems mentioned above, estimate potential coding capacity of the virus, and assign parts of the genome to specific proteins. We also hoped to use the virus to elucidate control signals used in eukaryotic cells since it seemed reasonable to suppose that it might mimic its host.

An additional purpose in sequencing polyoma virus DNA was to compare the genome with that of closely related monkey and human viruses, simian virus (SV) 40 and BK virus. Much comment has been previously made of the similarities between the mouse (polyoma) and monkey (SV40) viruses. Although their hosts are different, the overall organisation of their DNAs has appeared to be remarkably similar². This conclusion has been based primarily on the alignment of their respective messenger RNAs and their origins of replication relative to their DNAs, and on studies with a comparatively small number of mutants. However, attempts to hybridise the DNAs from the two viruses with each other in stringent conditions has revealed very little homology between the molecules.

The DNA sequence data, considered together with existing data on the viral proteins and mRNAs, now allow a more detailed description of the polyoma virus genome in functional terms. They also provide a predictive capacity not hitherto available for the virus. Moreover, a considerable homology with SV40 over about 80% of the genome is revealed, but large differences appear in the remaining 20%. These differences may be especially relevant since they appear to involve a transforming function⁴ or regions which appear to have regulatory functions.

General features of polyoma virus DNA

The A2 (large plaque) strain of polyoma virus is composed of 5,292 base pairs. The DNA sequence is given in Fig. 1 and regions of special significance are listed in Table 1. The DNA contains 47.3% guanine–cytosine base pairs. An earlier estimate of this value, based on analysis of restriction endonuclease fragments, gave 47.0% (ref. 5). More than 90% of the viral DNA appears to be used in coding for proteins. The corollary to this is that somewhat less than 10% of the sequence may serve other functions, probably involved with control and regulation of viral expression. Data available at present suggest that a part of the sequence between the viral origin of replication and the initiation signal for early proteins may be dispensable⁶, but there are no corresponding data about the sequence which lies between the origin of replication and the initiation signal for late

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 CAGACCGTAG GTCTCTGAT TTTAATCATC TCACATGGGG GTCCGCTGAT AATTTCTCTCT CTAAGCTTGT GATTGCAAGA GTCTCTCTCT ABGCTAAGG AACTTGGCTC CTCCGATGAT CTACTTACC
 2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280
 TAGCAGGTGT CCGTGGGATC CAGTGTCTTT TAGAGGACTT TCCTCAAAAC CTGTTTAAAG TGCTTAAGCT GCTAACAGAA AATGTGCCAA AACGACGCAA CATACTTTTT AGAGGACGAG
 ATCGTCCGCA GCGAACCATG GTCACAGAAA ATCTCTGTA AAGAGTTTGG GACAATTTCT ACBAATTTCT CBAATTTCT TACACGGTCT TTGCTGCTGT GTATGAAAGA TCTCTGCTGT
 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
 TTAATTCAGG AAGACGCGCG CTAGCAGCGC GCGTATTATG CCTGTATAGA GCGAAGTCTG TCAACATATA TTGCGCTGCA GATAAATCTA CTTTGTAGCT TGGTGTGBCA CAGGACGAGT
 AATTAGTCTC TTTCTGTCGG GATCGTCCGC GCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT CCGAATTAAT
 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520
 TTGTGCTGCT TTTTGAAGAT GTAAAGGCTT AATAGGCTT GAACAAAGAA CTGACAGAGC GAGTGGGCTG GATTAAGTCTA GATAATCTCA GACTACTCTA GATTAAGTCTA GATTAAGTCTA
 AACACAGAGC AAAATCTCTA CATTTCGGA TTTATCGGAA CTGTGTTGTT GAGTGGGCTG GAGTGGGCTG GAGTGGGCTG GAGTGGGCTG GAGTGGGCTG GAGTGGGCTG GAGTGGGCTG
 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
 ATCTAGAGAA AAGCAGAGC AACAAGAGT CACAAGTCTT TCCACCTGCT GTGTGTGAGA TGAATGAAAT TCTCTACCA CAACAGTAT GGGCCCGGTT TCACATGAGT TTGGATTAAT
 TAGATCTTTT TTCTGTTGCG TGTGTTTCCA GTGTGTGAGA AAGTGGGAGA CACACATGAT ACTACTTAT ATAGGATGAGT GGTGTTGAGT CCGCGGCCAA AGTGTAGCAG AACCTTAAGT
 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760
 CCTGCAAAAC CCATCTGCTG CAATCTCTTG AAAAGTGTGA ATTTTGTGAA AGGGAAGAGA TTAATCAGAG TGGAGATACC CTTGCCCTAT TACTCATAT GAATTTTCACT CAGATGTAT
 GAGCGTTTGG GGTAGACCGG GTTAGAGAAC TTTTACACCT TAAAGACGTT TCCTTTCTCT AATAGTCTCT ACCTCTATG GAAACGGGATA ATGAGTATAC CTTAAAGTGA AGTCTACATA
 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880
 TTGATCTGTA TATTCAGAGG CTGTGAGAGG AGGTTGCTGA CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT
 AACTAGGACT ATAAGTCCCA GACACTTCC TCCAAGCAGT GGTCAACAGA TCACTACAGA GATGTGCAAA CAAACACTA GATTGAGAT TATGAAACAT TACAGTGTCT TCCGCTGCTG GGGAACTTCC
 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
 ACATATGTGA TATAGCTGAA TACAGATGTT ATTGAATAAA CATTAAATTA CAGGAAGATC AGTCTTTGTT TTTCCAAAGT GGTCAACATA CCGGCTGCTA TCAAGGTCCT CCGGATACAG
 TGTATACACT ATATGACTT ATGTGTCAAA TACTTATATT GTAATTAAGG GTCTTTATG TCAAGAAACA AAGGTTTCC CCAAGTGTAT CCGGCTGCTA AGTCCAGGAG GGGCATGTCC
 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120
 TTGATCTGCA TCATACACTA TAACCTCTCT TACCTGGGTT TTCTCCCTCT CCAATGGGTT GCGCTGCACT TGGGGAGGCA TGTGTTGAA AAGGGAACCT ATGAGGAGAG CCATGGGATA
 AAGTCAAGGT AGTATGTGAG ATGGAGGAGG ATGAGCCCAA AAGAGGGGAA GGTACCAACA CCGGACGTGA ACCCCCTCT ACACAACTT TTCTCTGAA TACTCTCTCT GGTACCTAT
 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240
 GGGATTTTTT ACCCATCTTT TTCTCAGGCT GATTTTGAAA TATCTGGGAA AGCCCTTCTC CAGTGTGCTG CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT CCAAGTGTGCT
 CCTTAAAAAC TGGGTAGGAA AAGAGTCCCA CTAAGACCTT ATAGACCTTT TCGGAGAGAG GCTACACTAG TGTATCAAA AACTTTGAGA GGTCTGGGTA TATAGATGAT TCCTTCTCAT
 3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360
 TAGGCCCTCT CTTTACAGTA GGGGCGCCAC TCCATTTTCA TCTAGAGGCA CAGTGTGAGA GGTGTTTGTG AACTGACAGA CBBGTTGAGT TGTGTTGCTT CCAAGTGTAT TGGCAAGATA
 ATCCGGGAGA GGAATTTGCT CCGCGGGTGT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT AGGTAAGTCT
 3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480
 CCTTGTGATT TCATTTTTTT CTGATCTGAG ATGCCAGATT TCAACTGAGT ACATTCGCT CTTATCCAGC TTGGCTTTGG TAATTTGAT CAGGACTGAG TCCTTTGTA CAGTGTCTCT
 GGAACACAGG AGTAAAGAAC GACCTAGACC TACGCTCTAA AGTTGACCTA TGTAGGCGAG GAATAGGCTG AACCAGAGC ATTAACCTAA GTCTGTGAG AGAAACACT GGTACAGGAA
 3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
 CTTTGTGATT GTTTTGAATG TTACTACDCC TTCTTCTCTG TATTTTGTTC TGGCATCTGT CACAAGTCTC TGGAGGTGCG GCGGTTTCCC GCGGAGGAGG CCGGAGGAGG CCGGAGGAGG
 GAAACACTAA CAAACATAAC AATGATGGGG AAGAGAGAAC ATAAACCAAG ACCGTAGACA GTGTTCAGGG ACCCTCAGTT CCGCAAGGAG CCGGAGGAGG CCGGAGGAGG CCGGAGGAGG
 3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720
 TTCCACTGGA GTGGAATCTT CTTTGTGTTT ACTGAATCTT TGGGTTTTGT TGAACCCATG CACATCTAAC AGTGAAGTCT TCACTCTGCT CCGGAGGAGG CCGGAGGAGG CCGGAGGAGG
 AAGGTGACTT CACCTTAAGG GAAACACAAA TGACTTAGAC ACCCAAAACA ACTTGGGATG GTGATGATG TCACTCTGCT CCGGAGGAGG CCGGAGGAGG CCGGAGGAGG CCGGAGGAGG
 3730 3740 3750 3760 3770 3780 3790 3800 3810 3820 3830 3840
 TTGATGGGTT TCACAGGTGA GGTCTCTATT GAGGCTAGGG AAGCTGAGT TTGCTCATCT CCAATGTGGA AGTGTATTAT TTCGGGAGT CCACGTATCT GATGTAGGCA AATTAATCCC
 AACATCCCAC AGTGTCCACT CCAAGAGTAA CTCCGTACCC TTGACCTGGA AAGGATATGA GGTACACCTT TCACATAATA AABCCCTCTA GGTGATGAT CTACATCGGT TTAATTAGGG

Fig. 1

3850	3860	3870	3880	3890	3900	3910	3920	3930	3940	3950	3960
TCBCTCCAA	CCATAGTATT	GCCCTCCCTC	TGTTAGGCTT	TCAGGGGTGG	GTGGCTGCC	CATTCTGGGG	TTGAGAAAG	CTTCTATTTC	TGTACACATG	TCTGGCCCTG	TCACAAAGTC
AGACGAGGTT	GGTATCATAA	CGGGAGGGAG	ACAATCCGAA	AGTCCCAACC	CACCGACGGG	GTAAGACCCC	AAGTCTTTTC	GAAGATAAAG	ACAGTGTGAC	AGACCCGGAC	AGTGTCCAG
3970	3980	3990	4000	4010	4020	4030	4040	4050	4060	4070	4080
CAGCACTCC	ATACCCCTTC	TAATAAGCAG	TTTGGGACAT	GGTGCAGGCT	TTGAGCAGCG	CTTTGTACAT	TTTGTCTGCG	ATTTAGAGAC	GCCGCTTTTC	CTTTTGGGGG	CCCATCTTCCT
GTCGTGGAGG	TATGGGGGAA	ATTATTCGTC	AAACCTTTGC	CCACGCCGAC	AACCTGTCCG	GAACATGTA	AAACABAGCG	TAAATCTCTG	CGGGCAAAAA	GAACACCCCT	GGTAGAGGA
4090	4100	4110	4120	4130	4140	4150	4160	4170	4180	4190	4200
CTATGACTGT	TGCCCCAGTA	GGTGTGATAT	CACCGTACAG	CCCTGAAATT	AAAGGAAAGT	TCCAGTCAGG	AGTGACTCTT	TGGTGGGACA	CCACCTGGGG	CTGATAGAAC	TTTATCACTT
GATACCTGAC	ACGGGTTCAT	CCACACTATA	GTGGCATGTC	GGGACTTTAA	TTTCTCTTCG	AGGTGAGTCC	TCACCTGAGAA	ACCACCTCTG	TGGTGGACCC	GACTATCTTG	AAATAGTGA
4210	4220	4230	4240	4250	4260	4270	4280	4290	4300	4310	4320
CACCTGACTC	ATCCGTGATA	TGTGCTGCAG	GGGTAGGCC	ACCATTTCCC	ATGCTCCCTT	CTATGCGATT	AAAGAGTGCT	CTCCTCTGTG	GTGGATTAGG	ACCTAGTTGC	CTATAATAGT
GTGGACTGAG	TAGGACATAT	ACACGACGTC	CCCATCCGGG	TGGTAAAGGG	TACGAGGGAA	GATACGCTAA	TTTCTCAGCA	GAGGAGACAC	CACCTAATTC	TGGATCAACG	GATATTATCA
4330	4340	4350	4360	4370	4380	4390	4400	4410	4420	4430	4440
CACCTAGTGA	TGAGTACCTT	GAGCTCGCTG	ATGAGGACAC	TCTGTTGATT	GCATCTATGG	CTGACTGAGG	AGCATTAGAA	ACCACCCACC	GGGTGTTTTT	AAGTAGCCTA	GCTAGGCCAT
GTGAATCCTC	ATCATGAGGA	CTCGAGCGAC	TACTCCGTGG	AGACAACATA	CGTAGATACC	GACTGACTCC	TCGTAATCTT	TGGTGGGTGG	CCACAAAAAG	TTTCTCGGAT	CGATCCGGTA
4450	4460	4470	4480	4490	4500	4510	4520	4530	4540	4550	4560
CCAGGAATGT	ATGTGCTCTG	CTTACAGTGA	GTTCCTTTAC	AGTCTCTTAC	AGTCTGTGTT	GTGTTCTCTG	CACAAACCAT	TGCCACACAT	ATCTTCCCAT	GGAAATGAAG	AGGCGCATGGC
GGTCTTTACA	TACACAGACA	GAATGTCAAT	CAAGAGAAATG	TCGAGGAAGG	TCAGACACAA	GCTTTCTGTA	CACAAAGGAC	GTGTTGTTAA	ACGGTGTGTA	TAGAAGGGTG	CTTACTTTCA
4570	4580	4590	4600	4610	4620	4630	4640	4650	4660	4670	4680
CCCAATCATT	TACTACATTT	AGAGCATGAG	CAAACTGATT	AACCTCTGGG	AAGTATATAT	CGAGAAAGGG	TGGATCCCGC	CTAGGTATCA	ACGCCATATT	TCTATTATCA	GTAGGAGACCT
GGGTAGTAC	ATGATGTAAA	TCTGCTACTC	GTTCGACTAA	TTGAGGACCC	TTCATATATA	GCTCTTCCCG	ACCTAGGGGG	GTACCATAGT	TGCCGTATTA	AGATAAATGT	CATCCCTGGA
4690	4700	4710	4720	4730	4740	4750	4760	4770	4780	4790	4800
CTTCGTTTGT	TAGGTACCGC	TGATTTCCCTA	GGGAAATATG	AGAGGCCACC	TGAAGTGTCT	GCATCAGCCA	TATAGCCCCC	GCTGTTCCAG	TTACAAACAC	AGGCACAGTA	CTGACAAACC
GAAGCACAC	ATCCATGGCG	ACATAAGGAT	CCCTTTATCA	TCTCCGTGGA	ACTTGAACAGA	CGTAGTCGGT	ATATCCGGGG	CGACAGGCTG	AATGTTTGTG	TCCGTGTCAT	GACTGTTTGG
4810	4820	4830	4840	4850	4860	4870	4880	4890	4900	4910	4920
CATACACCTC	CTCTGAAATA	CCCATAGTTG	CTAGGGCTGT	CTCCGAACCT	ATTACACCTT	CCAAAGTCAG	AGCTGTAAAT	TGCGCATCAA	GGGCAAGCAG	GGCTTCTCCA	GATAAATATG
GTATGTGGAG	GAGACTTTAT	GGGTATCAAC	GATCCCGACA	GAGGCTTGAG	TAATGTGGGG	GGTTTCACTG	TCGACATTAA	AGCGGTAGTT	CCCGTCCCTC	CCGAAGAGGT	CTATTTTATG
4930	4940	4950	4960	4970	4980	4990	5000	5010	5020	5030	5040
CTTCTGCCGA	GAGTCCCGTA	AGGGTAGACA	CTTCAGCTAA	TCCTTCGATG	AGGTCTCATG	GAATAGTCAG	TGCGGCTCCC	ATTTTGAAAA	TTCATTACT	TGATCAGCTT	CAGAAGATGG
GAAGACGGCT	CTCAGGGCAT	TCCCATCTGT	GAAGTCGATT	AGGGAGCTAC	TCCAGATGAT	CTTATCAGTC	ACGCCGAGGG	TAAAGCTTTT	AAGTATGATG	ACTAGTCGAA	CTATTTCTACC
5050	5060	5070	5080	5090	5100	5110	5120	5130	5140	5150	5160
CGAGAGGAGC	CCAGACAGAT	AATTTTCTCT	CCGACTCTTA	AAATAGAAAA	TGTCAGATGA	GTAAAGTCAG	AGGTGATGAC	ACTGACGCGA	CTGGGCGTGC	GACATCCCTC	TTTAAATGAT
GCTCCTCCGA	GGTTGTGTCA	TTAAAAAGAG	GGCTGAGATT	TTTATCTTTT	ACAGTTTCAGT	CAATTCGCTC	TTCACTGAT	TGACTGCGTC	GACCGGCGAC	CTGTAGGAGA	AAATTAATCA
5170	5180	5190	5200	5210	5220	5230	5240	5250	5260	5270	5280
TGCTAGGCAA	CGCCCTCCAG	AGGGCGTGTG	GTTTTGCAAG	AGGAAGCAAA	AGCCTCTCCA	CCGAGGCTCA	GAATGTTTCC	ACCCAATCAT	TACTATGACA	ACAGCTGTGT	TTTATTAGAT
ACGATCCGTT	CGGGAGAGTC	TCCCGCACAC	CAAAACGTTT	TCCTTCGTTT	TCGAGAGAGT	GGGTCCGGAT	CTTACAAGGG	TGGGTTAGTA	ATGATAGTGT	TGTCGACAAA	AAAAATCAT
5290	8	18	28	38	48	58	68	78	88	98	108
TAAGCAGAGG	CC										
ATTGCTCTCC	GG										

Fig. 1 The total sequence of polyoma virus DNA (A2 strain ref. 45), which contains 5,292 base pairs. The top strand contains a sequence with the same polarity as early mRNAs and the bottom strand contains a sequence with the same polarity as late mRNAs (ref. 46). The location of the eight *HpaII* restriction endonuclease sites are indicated (for example, 3/5, 5/4). Using the convention previously adopted¹¹, the centre of the *HpaII* 3/5 junction (CC'GG) has been designated zero and nucleotides are numbered proceeding in a clockwise direction on the physical map (see Fig. 3). This *HpaII* site probably includes the viral origin of replication, and such numbering allows easy comparison with the SV40 sequence of Fiers *et al.*⁷, or the sequence as renumbered by Buchman *et al.*⁴⁷. The location of the 11-base pair deletion found in the A3 strain of the virus⁴⁵, between nucleotides 43 and 55 (ref. 11), is indicated on the sequence by arrows (▼). The probable ATG initiation codon for the early proteins (position 173) and for the late proteins VP1(4074), VP3 (4657) and VP2 (5002) are shaded. Other sites of known or postulated significance are given in Table 1 and Fig. 3. Most of the sequence was derived using either the chemical sequencing procedure of Maxam and Gilbert⁴⁸, or the dideoxy method of Sanger *et al.*⁴⁹. Over parts of the genome, both methods were used. Moreover, for most of the genome, both strands were sequenced. Details are given elsewhere^{11,12,26,27}. Over 95% of the sequence was derived from studies on a single preparation of polyoma virus DNA, using a stock of A2 strain virus made from an isolate which was twice plaque purified.

proteins. With polyoma virus, for example, there are no extensive sequence duplications such as are found in the late region of SV40 (refs. 7, 8). For further consideration, the DNA is divided into 'coding' and 'noncoding' regions, and specific features are discussed in the relevant sections.

The coding regions

The early coding region extends from about 74.0 to 25.5 units clockwise on the physical map of polyoma virus DNA (ref. 9) and consists of about 2,700 base pairs. Assuming a co-linear relationship between DNA, RNA and protein, this amount of DNA would contain the coding capacity for proteins with a total molecular weight (MW) of about 100,000, and any splicing of the viral RNA molecules would reduce the estimate. However, the three known early proteins of polyoma virus, the small t, middle T and large T antigens, have been assigned combined MWs of about 175,000 (see ref. 10 and Table 2). The T antigens appear to be genuine virally coded proteins, so it is obvious that the virus needs extra coding sequences. From the DNA sequence, this appears to be accomplished by using more than one coding frame in a particular region of the DNA, that is, proteins are encoded in overlapping genes¹¹. The coding potential of the early region is illustrated schematically in Fig. 2a. If the DNA is divided into its three coding frames and a solid block is placed within a frame whenever one of the triplet codons TAA, TAG or TGA (the putative termination signals) appear in the sequence, it is apparent that between 74 and 85 map units there is only one open coding frame. This has been designated frame 1. This frame is then closed, and between about 85 and 99 map units the other two frames (frames 2 and 3) are open. From

99 map units to the end of the early region, only one extensive frame (frame 2) is open, although short open areas are found in the other two frames. Thus, coding throughout the early region requires frameshifts^{12,13}. These data are summarised in Fig. 2a, and protein assignments allowed by these and other data¹⁴ are illustrated in Fig. 2b. From other studies of the proteins themselves, studies on mutants, and by analogy with SV40, frame 1 is believed to code initially for all three of the early proteins and they are thought to share common N-termini¹⁵⁻¹⁷. The bodies and C-termini of middle T antigen and large T antigen are encoded in frames 3 and 2, respectively. The C-terminus of small t antigen may lie in frame 1. Additional data taken from the DNA sequence (Fig. 1) which predict the location of the termination codons for each protein, postulate splices which occur in the mRNAs to produce the relevant proteins, and suggest the location of other significant or novel sites on the DNA are summarised in Table 1a. Some of these data have been published elsewhere¹¹⁻¹³.

One interesting aspect of the DNA sequence is that there is a strong bias in the distribution of termination codons. Whereas the termination signals TAA, TAG and TGA appear with about equal frequency through most of the early region in frames which are probably not being used for coding, this is not so for the region in frame 1 between about 86 and 95.5 map units. In this region, the only termination codon found in the DNA sequence is TGA. Were an occasional 'readthrough' of this signal to occur, a protein with MW of about 40,000 could be made entirely from frame 1, allowing, that is, for a suitable splice in the mRNA to remove the termination codon triplet (TAG) encoded in the DNA in this frame at position 806 (85.7 map units). This point is made not only because of this unusual

Table 1 Landmarks on polyoma virus (A2 strain) as suggested by DNA sequence

<i>a, Early region (from about 1 to 2,920 nucleotides, see top strand, Fig. 1)</i>				<i>b, Late region (from about 5,292 to 2,908 nucleotides, see bottom strand, Fig. 1)</i>			
Nucleotide no.	Sequence	Significance	Refs	Nucleotide no.	Sequence	Significance	Refs
5263-45 (5267-18)	See Fig. 1	Region around viral origin of replication (hairpin loop)	11, 42, 52	5,283-5,268	TTAATACTA ₈	A, T-rich region near viral origin of replication	11
87-97	GCCTTGGAGGC	Single <i>Bgl</i> I site (72.2 map units)	11, 52	5,155-5,148	TTAAAAGA	'Hogness box'—potential late promoter	26
5078-5085 5151-5158 120-127 272-279	TTAAAATA TTTAATTA TATAATTA TATAAGCA	'Hogness boxes'—potential early promoters ≥ 75% homology with canonical TATAAATA)		5,094-5,086	GACATTTTC	Possible location of 5'-ends of late mRNAs	32
54-58 142-146	CTTCC CCTTCC	Possible ribosomal binding sites	11, 53	5,023-5,018 5,081-5,076	TCAA/GTA TTAAG/A	Possible 'leader to leader' splice which gives rise to tandem repeats in late mRNAs	26, 31
173-175	ATG	Probable initiation codon for known early proteins (T antigens)	11, 16	5,091-5,078 5,066-5,060 5,012-5,001	AT ₄ CTAT ₄ AA AAAATTA AAT ₄ CAAA	AT-rich regions in noncoding sequence	26
512-698	See Fig. 1	Deletion in hr-t mutant, NG-18	54, 55	5,037-5,031	TCTTCTG	Possible ribosomal binding site	26, 53
406-412 790-795	CCAG/GTA TACAG/G	Possible splicing signals for large T antigen messenger	12, 14	5,021-5,026	TGATCA	Single <i>Bcl</i> I site (65.4 map units)	
743-749 790-795	CCTAG/GTA TACAG/G	Possible splicing signals for small t antigen messenger	12, 14	5,023-5,018 5,019-5,014	TCAA/GTA GTAAG/T	Possible 'leader to body' splice for 19S late mRNA (alternative, no splice)	26, 3
Either 762-767 790-795 Or 743-749 804-809	GCGG/GTG TACAG/G CCTAG/GTA CCTAG/A	Possible splicing signals for middle T antigen messenger	12, 14	5,023-5,018 4,712-4,705 5,023-5,018 4,127-4,122 5,002-5,000 4,657-4,655 4,074-4,072 4,632-4,637	TCAA/GTA CCTAG/G TCAA/GTA TCTAG/G ATG ATG ATG GGATCC	Possible splice for 18S late mRNA Possible splice for 16S late mRNA Initiation codon for VP2 Initiation codon for VP3 Initiation codon for VP1 Single <i>Bam</i> I site (58.0 map units)	26, 32 27 25-27
705-808	See Fig. 1	Minimum region containing at least one termination codon in each coding frame	11	4,045-4,043	TAA	Termination codon for VP2/VP3	26
806-808	TAG	Proposed termination codon for small t antigen	12, 14	4,074-4,046	See Fig. 1	Overlap region between genes coding for VP2/VP3 and VP1	26, 27
1,158-1,176	(GGA) ₆ G	Unusual triplet sequence repeat	11	2,925-2,923	TAA	Termination codon for VP1	27
1,215-1,219	G ⁺ GGGG	Possible location(✓) of 5-MeC (on complementary strand)	11	2,913-2,908	AATAAA	Possible processing signal for late mRNAs	12, 27
1,476-1,481	AATAAA	Possible processing signal for unknown early mRNA	11, 14				
1,498-1,500	TAG	Proposed termination codon for middle T antigen	11				
1,560-1,565	GAATTC	Single <i>Eco</i> RI site (0/100 map units)	5, 11				
2,222-2,776	See Fig. 1	Region of very high homology with either SV40 DNA or its predicted T antigen sequences	12, 13				
2,584-2,785	See Fig. 1	Long open reading frame beginning with ATG. Could code for a small protein	12				
2,913-2,915	TGA	Proposed termination codon for large T antigen	12				
2,915-2,920	AATAAA	Possible processing signal for early mRNA(s)	12, 14				

feature of the sequence, but also because a protein of MW 39,000, although not yet completely characterised, is frequently found together with the other polyoma virus early proteins¹⁵. Similarly, it is interesting to speculate that a second large T antigen observed on polyacrylamide gels, which has an MW

slightly greater than usually found¹⁸, could be due to read through of the UGA termination codon for large T antigen. Precedence for this suggestion appears in studies on the phage Q β where a low level of readthrough of the UGA codon for the coat protein occurs to produce a longer, functional protein¹⁹. An alternative, and attractive, explanation is that TGA may not always specify a termination codon. In two mitochondrial systems, it has recently been suggested that UGA, instead of functioning as a termination codon, may code for tryptophan^{20,21}. If either readthrough of UGA occurred or in polyoma virus UGA were a coding triplet, it is clear that this virus could specify more proteins than those which are known at present.

The late coding region extends from about 65 to 25.5 units anticlockwise on the physical map of polyoma virus DNA and consists of about 2,100 base pairs. This is enough information to code for proteins of approximate total MWs 77,000 in a co-linear fashion. The three known late proteins (VP1, VP2 and VP3) of polyoma virus have been assigned combined MWs of about 105,000 (see Table 2). However, it would appear that although they seem to be derived from independent mRNAs (ref. 22), all the amino acid sequences present in VP3 are also present in VP2. Therefore, the late proteins essentially require coding information for an MW of only about 80,000, which roughly corresponds to the prediction from the DNA sequence.

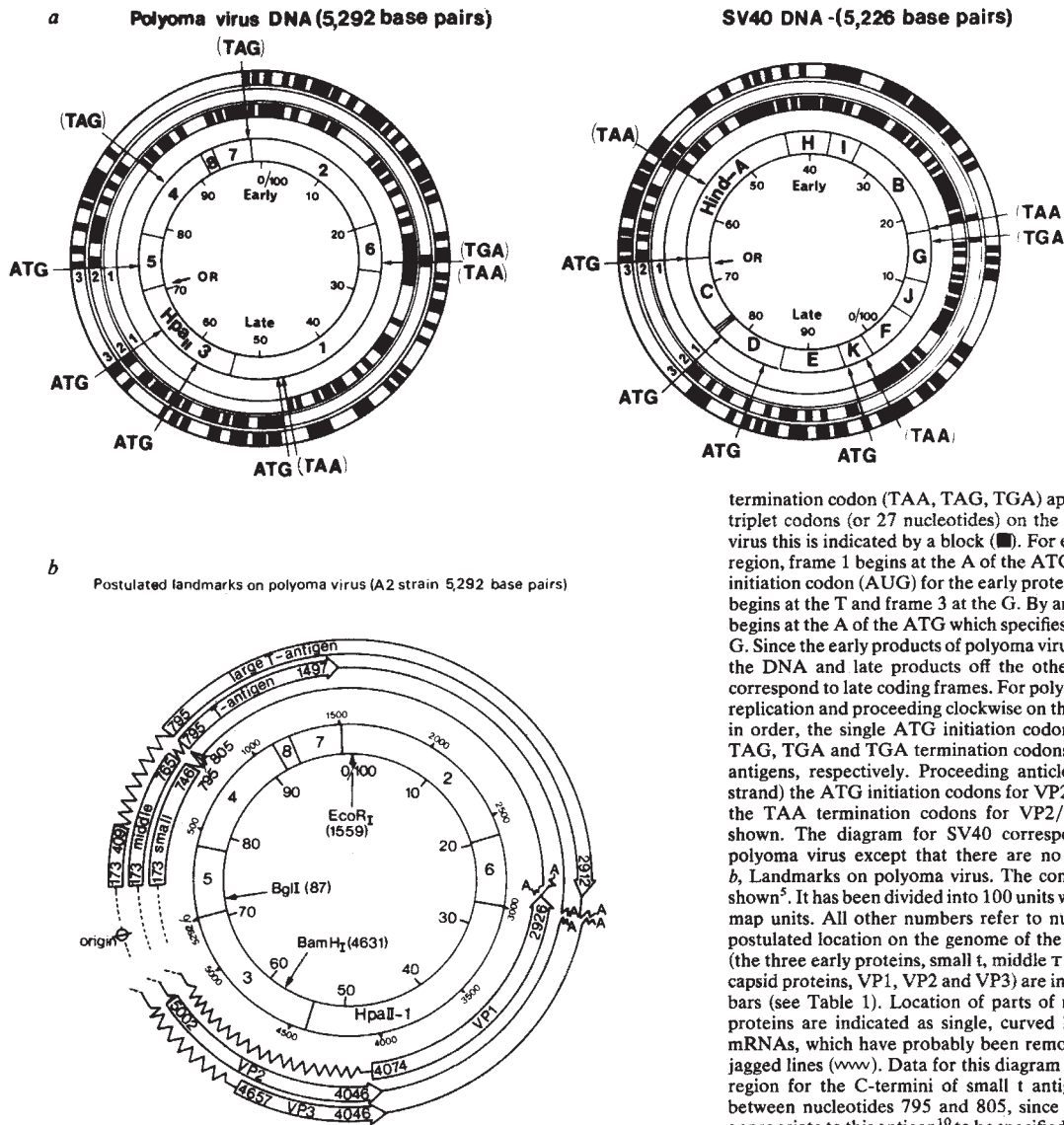


Fig. 2 a, A schematic diagram which divides the DNAs of polyoma virus and SV40 into three coding frames relative to their respective conventional physical maps. For polyoma virus, there are eight *HpaII* fragments and the map is divided into 100 units with the *EcoRI* site at 0/100 map units⁵; for SV40, there are 13 *HindIII* and *HindIII* fragments⁷ and the map has been similarly divided with the *EcoRI* site at 0/100 map units. The conventional SV40 map (ref. 50) has been reorientated to allow a direct comparison with polyoma virus to be made. In each case, the viral origin of replication (OR) is also indicated. Whenever a

termination codon (TAA, TAG, TGA) appears in the sequence within nine triplet codons (or 27 nucleotides) on the appropriate coding strand of the virus this is indicated by a block (■). For each virus within the early coding region, frame 1 begins at the A of the ATG codon which corresponds to the initiation codon (AUG) for the early proteins; consequently, coding frame 2 begins at the T and frame 3 at the G. By analogy, in the late region, frame 1 begins at the A of the ATG which specifies VP2, frame 2 at T and frame 3 at G. Since the early products of polyoma virus are transcribed off one strand of the DNA and late products off the other⁵¹, early coding frames do not correspond to late coding frames. For polyoma virus, starting at the origin of replication and proceeding clockwise on the outside of the circle, are shown, in order, the single ATG initiation codon for the early proteins, and the TAG, TGA and TGA termination codons of small t, middle τ and large T antigens, respectively. Proceeding anticlockwise (and from the opposite strand) the ATG initiation codons for VP2, VP3 and VP1 respectively, and the TAA termination codons for VP2/VP3 and VP1, respectively are shown. The diagram for SV40 corresponds in organisation to that of polyoma virus except that there are no signals for a middle τ antigen. **b,** Landmarks on polyoma virus. The conventional *HpaII* physical map is shown⁵. It has been divided into 100 units with the single *EcoRI* site at 0/100 map units. All other numbers refer to nucleotide numbers in Fig. 1. The postulated location on the genome of the six known virally coded proteins (the three early proteins, small t, middle τ and large T antigens, and the late capsid proteins, VP1, VP2 and VP3) are indicated in the appropriate curved bars (see Table 1). Location of parts of messenger RNAs not coding for proteins are indicated as single, curved lines. DNA sequences absent in mRNAs, which have probably been removed by splicing, are indicated by jagged lines (www). Data for this diagram are found in Table 1. The coding region for the C-termini of small t antigen is assigned to the sequence between nucleotides 795 and 805, since this allows a protein with a size appropriate to this antigen¹⁰ to be specified. Note that bodies of middle τ and large T antigens are encoded in two different frames as are VP2/VP3 and VP1. There is an alternative splice for middle τ antigen at a position between 747 and 800 map units; S₁ nuclease mapping data suggest that this is probably the correct one¹⁴. The coding frames used for each protein are shown in a.

The sequences of the N-termini of the three late proteins have been determined²⁵. It is thus possible to position them accurately on the DNA sequence. As above, the coding potential of the late region sequence is illustrated schematically in Fig. 2a. It can easily be seen that proceeding anticlockwise on the physical map from the origin of replication, only one reading frame (designated frame 1), between about 65 and 47 map units, contains no termination codons. This frame is then closed, and between about 46.3 to 25.5 map units only frame 2 is open for any extensive region. Thus, coding over the entire late region requires two reading frames. There are other unblocked regions within each frame over the entire late region which could be used for coding small polypeptides although no proteins other than the three mentioned above are known. The protein assignments on the genome are shown in Fig. 2b and the data are summarised in Tables 1b and 2. In coding terms the late region generally contains no surprising features and an analysis of it has been given elsewhere^{26,27}. As in SV40 (refs 7 and 8), the C-termini of VP2/VP3 overlap the N-terminus of VP1 although the overlap in polyoma virus is less extensive than that predicted for the former (see Fig. 2).

The codons used by polyoma virus are given in Table 3. What is immediately apparent is the small use made of any codon which incorporates CG. Although CGN and NCG are occasionally used, their use is well below average, whereas CG when it appears between codons (NNC-GNN) occurs with average frequency (data not shown). This result is consistent with earlier studies on the dinucleotide composition of polyoma virus DNA²⁸. A surprising feature is the low use of the codon AUC for isoleucine or GUC for valine, and to a lesser extent CUC for leucine, although the same discrimination against NUC codons is observed with SV40 (refs 7 and 8).

In summary, polyoma virus contains more coding information than a co-linear relationship between DNA, RNA and proteins would suggest. In several parts of the genome, notably in about half of the early region, more than one coding frame is used. The DNA sequence allows data from studies on viral mutants, mRNAs and proteins to be correlated. It should be emphasised that the latter data are by no means extensive and it is possible that our ideas about the coding potential of polyoma virus will later appear too restricted. The sequence seems to imply this. As a by-product of these studies, which allow the DNA sequence to

be aligned with the (known) polyoma virus proteins, we should now be able to construct mutants to test the current model for the functional viral genome, as illustrated in Fig. 2b.

The non-coding or control regions

There appear to be two noncoding regions in the polyoma virus genome interspersed between coding regions. One lies entirely within the early region, probably between nucleotides 746 and 795 (Fig. 1), and the other lies around, and includes, the viral origin of replication. The presumption with the former is that it contains the information which regulates splicing events so that they occur in such a way as to produce functional messengers for the three early proteins, the small t, middle T and large T antigens. The dinucleotides GT and AG are thought to be important splicing signals²⁹ and the coding strand of this region, as well as the sequence which immediately precedes it, has a number of these dinucleotides. Between positions 724 and 794 there are seven GT and five AG dinucleotide sequences. Note that the entire region under consideration can adopt a moderately stable secondary structure which resembles a clover-leaf (Fig. 3a). The hypothesis that a GT dinucleotide lies at the 5'-side of an intervening sequence and an AG lies at the 3'-side seems now to be supported by data from a wide variety of sources (for review, see ref. 30). The assumption can therefore be made that any one, or all of these dinucleotides, could theoretically be used in the recognition events that lead to RNA splicing, but it is not known if structures such as that shown in Fig. 3a are important. Although the preferred splicing sites for the three proteins, as deduced from S₁ single-strand specific nuclease studies¹⁴, are given in Table 1a, it can be envisaged that through either error or some subsequent selection procedure, other sites might be used during the growth cycle of the virus. These could lead to a variety of messengers being produced which specify proteins with only slightly modified sequences¹². At the level of sophistication being used to study the viral proteins, this would not be noticed. Nonetheless, small changes in amino acid sequence at a crucial site in the protein could easily alter the tertiary structure and thereby the function of a protein. By such a route, a small virus could greatly extend its coding capacity. Whether this is the case with polyoma virus, or whether, because of other structural factors the virus uses only a minority of its potential splicing signals, is not known.

The second noncoding region must contain control signals for viral replication and for the initiation of transcription. It should also contain information for the correct splicing events which produce functional late mRNAs^{31,32}. This region extends from positions 172 downstream along the sequence given in Fig. 1 to, and including, position 5,003. Some of the nucleotides around the two *HhaI* restriction endonuclease sites at positions 85 and 96 (about 72 map units) are not essential to the virus⁶. Both these sites and the regions around them can be deleted, and the resulting mutants appear both to replicate and to transform cells with a facility which approximates that of the wild-type virus. Without further direct evidence, it may be assumed that the rest

of the sequence in this noncoding region serves some function. A number of sites of possible relevance to control and regulation of the virus are pointed out in Table 1. One of the most important is, of course, the viral origin of replication. It has been suggested that the origin probably lies around, and may include, the *HpaII* restriction endonuclease junction between fragments 3 and 5 (refs 5 and 11). The sequence from this region can form either a stable hairpin loop or an interesting four-stranded conformation¹¹. Moreover, it shows considerable homology with sequences from around the origins of replication of SV40 and BK virus. On the late side of the putative polyoma virus origin of replication, that is, within the 290 nucleotides that lie between positions 5,003 and 5,292 (Fig. 1), there are a number of AT-rich regions. These might facilitate the separation of the DNA strands through interaction with either an RNA molecule (see below) or a protein and allow the DNA to function at any given time essentially as a single-stranded species. Since the major mode of replication in polyoma virus is bidirectional and proceeds from a single site (or region) on each of the two strands, initial strand separation may be an important event in DNA replication.

The polyoma virus late mRNAs appear to consist of a non-coding 5'-'leader' sequence which, by some mechanism not yet understood, appears to be amplified, probably during transcription³¹. This sequence is attached to the main body of the mRNA in a process that involves (presumably) RNA splicing. The DNA sequence which corresponds to the late leader sequence appears to lie between nucleotides at positions 5,094 to 5,019. A part of this sequence, as deduced from a predicted ability to form base pairs, has a high degree of complementarity with the sequence which immediately precedes the initiation codon for VP3. That is, of the 46 nucleotides that lie between positions 5,092 and 5,046, 31 residues can form base pairs with the nucleotides that lie between positions 4,657 and 4,699. Moreover, part of the leader sequence can form a fairly strong secondary structure with the sequence which precedes the VP1 initiation codon²⁷. Whether these long-range potential structures have any significance, and, if so, whether the significance lies at the DNA level, with DNA exercising control over transcription by these means, or at the RNA level, with precursor RNA controlling its own splicing (and thus translation), or with the mRNA controlling translation by its own secondary structure, is not known. It seems unlikely, however, that their potential presence within the genome is fortuitous.

If it is assumed that the sequence around the *HpaII*-3/5 restriction endonuclease junction plays a part in viral DNA replication, and the sequence near the late coding region is of importance for the initiation of transcription, there are still about 150 nucleotides between positions 5,260 and 5,110 (Fig. 1) to which a function must be assigned. Within this region, the only assignment made (Table 1b) is to a potential promoter, that is, a sequence which resembles a so-called 'Hogness-box'. There is, however, a sequence which lies within this region which would allow the DNA, if transcribed, to produce an RNA which can adopt a cloverleaf configuration. This is illustrated in Fig. 3b. Such a structure was sought within the DNA because, as discussed above, it was apparent that if the UGA termination codon could be suppressed, a large amount of coding information would become available to polyoma virus. As some strains of *Escherichia coli* are known to contain a UGA suppressor, a tRNA^{Trp} (ref. 33), a search was made for a sequence in the virus which might specify a UGA suppressor. Since a viral gene specifying a tRNA might contain intervening noncoding sequences, such a search is not completely straightforward. One surprising result, however, was the considerable secondary structure found to be inherent in the DNA, and therefore to reflect structures which could exist in transcription products. The cloverleaf structure shown in Fig. 3b was one of the most stable structures observed and it could be formed from the nucleotides between positions 5,231 and 5,160. It does not, however, contain the appropriate anticodon for a UGA suppressor tRNA. The structure illustrated here as DNA has

Table 2 Calculated and measured molecular weights of polyoma virus proteins

Protein	Measured MW	(ref.)	Calculated MW*	Amino acids (no.)
VP1	45,000–48,000	(23, 24)	42,404	385
VP2	35,000	(24)	34,761	319
VP3	23,000	(24)	22,866	204
Large T antigen	88,000–100,000	(10, 56)	87,991	785
Middle T antigen	55,000	(57)	49,710	432
			(48,566)†	(421)†
Small t antigen	22,000–23,000	(56)	22,785	195

*Based on DNA sequence.

†Depending on which splicing signals are used to produce the mRNA (see Fig. 2b).

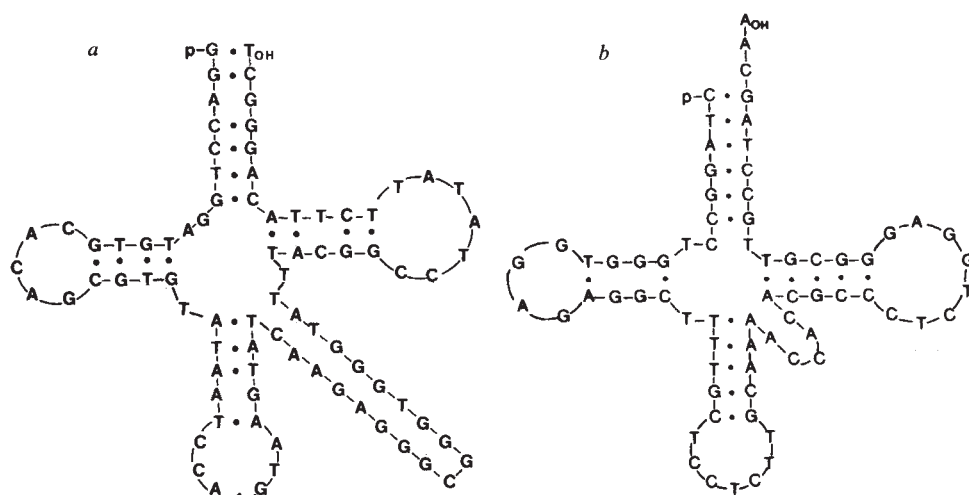


Fig. 3 *a*, Secondary structure of the DNA which lies in the early region of the genome between positions 715 and 799 (see Fig. 1). This region is of special interest because it appears to contain information which specifies some of the splicing signals in the early mRNAs^{12,14}. *b*, An unusual clover-leaf structure allowed by the sequence of the DNA in a noncoding part of the polyoma virus late region, using nucleotides which lie in the late strand between positions 5,231 and 5,160. (It is doubtful, however, that the left hand loop, as such, would exist since it is stabilised by only two base pairs.) The anticodon loop has a similar, but not identical, sequence to postulated ribosomal binding sites^{11,53} (see Table 1). An analogous kind of structure could be drawn for the complementary strand. This structure and the one above were found by a computer search using an unpublished computer program of R. Staden.

many, but not all of the features, of tRNA (for review see ref. 34). For example, it can be seen that the D-loop (left hand loop) could form hydrogen bonds with the T ψ C-loop (right hand loop). On the other hand, no tRNA has yet been described that has an anticodon loop composed entirely of pyrimidines (or on a corresponding structure from the other strand of the DNA, all purines). The comparison and contrast could be extended, but is of academic interest only unless it can be shown that polyoma virus codes for a tRNA. At present, it is not known whether the virus codes for any low molecular weight RNA molecules.

Apart from the question posed about viral tRNAs, nothing is known about the initial steps in viral DNA replication and transcription. It is conceivable that an RNA with a structure which corresponds to that shown in Fig. 3*b* might be involved in one or the other of these processes.

A comparison with SV40 and BK virus

It now appears that although much of polyoma virus resembles SV40, in some aspects the two viruses show profound differences. Since BK virus has been shown to be very similar to SV40 (refs. 35–37), much of what is said about the latter is presumably also true for the former.

Dealing first with similarities, the late coding regions in polyoma virus and SV40 have many features in common. Both viruses code for three late proteins which have similar sizes and appear to contain many homologous amino acids. Potential coding similarities between the two genomes themselves are illustrated in Fig. 2*a* and between their DNAs and predicted amino acids in Fig. 4*a* and *b* respectively. The latter also includes a comparison with BK virus. The greatest homology lies near the C-termini of the VP1 proteins. This conclusion, which is based on DNA sequences alone, is supported by the finding that the VP1 proteins of polyoma virus and SV40 are immunologically cross-reactive³⁸; none of the other proteins has been found to be related in a similar way. In the region which contains the overlapping genes for parts of VP2/VP3 and VP1 (see Table 1*b*), 19 of the 32 base pairs (60%) in polyoma virus are homologous with nucleotides found in the similar position in SV40 (ref. 26). Although a region which codes simultaneously for parts of two proteins might be expected to be highly conserved in related viruses, the homology is, in fact, no greater than that which exists in similar lengths of sequence in many other parts of the genomes which code for one protein only, notably, for example, in regions near the N-termini of the large T

antigens and the VP1 proteins. This may suggest that the sequence within the overlap is of little functional significance; on the other hand, it may emphasise the importance of retaining the sequence not only of the overlapping genes, but also in other regions in order to conserve diverse functions of the proteins. It has previously been argued, for example, that the quarter of

Table 3 Codons used in polyoma virus genes

		U		C		A		G	
U	Phe	42		26		Tyr	25	Cys	27
		25		17			25		17
	Leu	22	Ser	25	Ochre	1	Opal	1	A
		22		4	Amber	2	Trp	29	G
C		40		38		His	27		3
	Leu	28		31			19	Arg	10
		51	Pro	42		Gln	37		9
		45		10			43		11
A	Ile	31		37		Asn	45	Ser	27
		9		35			23		33
	Met	29	Thr	36		Lys	47	Arg	50
		44		5			43		22
G		31		47		Asp	50		23
	Val	11		24			41	Gly	28
		28	Ala	37		Glu	55		47
		33		9			61		34

These data are taken from the coding sequences for VP1, VP2, middle T antigen and the part of large T antigen encoded in frame 2 (Fig. 2*a*). (All sequences present in VP3 are present in VP2 (ref. 24). Most of the sequence in small t antigen is also present in middle T antigen (refs 11, 15, 16), as is the frame 1 sequence of large T antigen.) These data are tentative since neither the exact splicing to create the mRNAs for middle T and large T antigens nor the sequence coding for the C-terminus of small t antigen are known. However, the data should at least be a very close approximation of the codons used. The bias against the use of CG is notable in codons specifying Ser, Pro, Thr and Ala but less notable in codons specifying Arg. Overall, this bias is much less than is observed in the case of SV40 (refs 7 and 8).

large T antigen nearest its C-terminus may be important in viral DNA replication¹².

Over a large part (about 80%) of the early coding region, there is considerable homology between predicted amino acid sequences of two of the early proteins of polyoma virus and the two corresponding proteins (small t and large T antigens) of

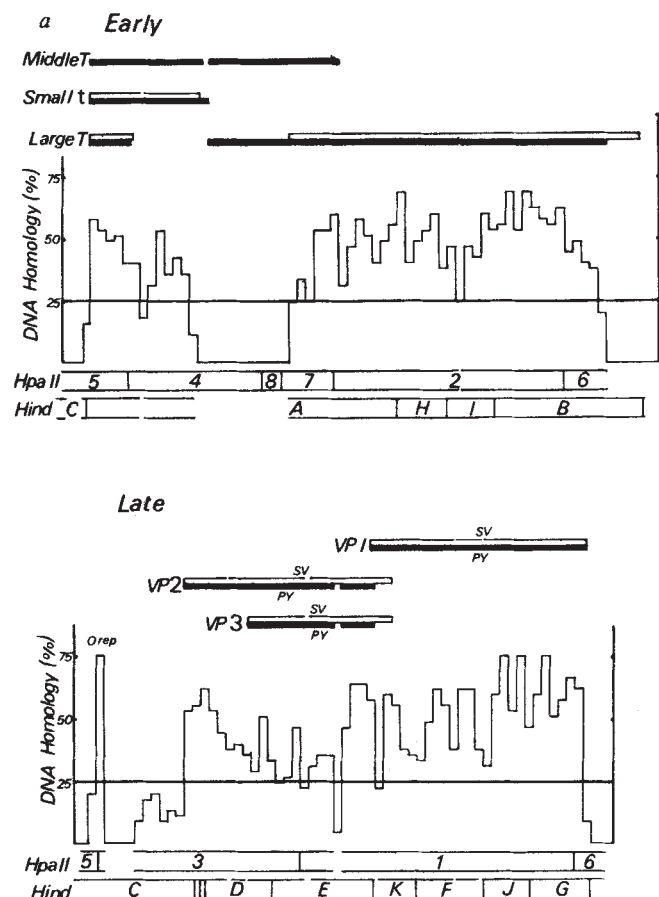
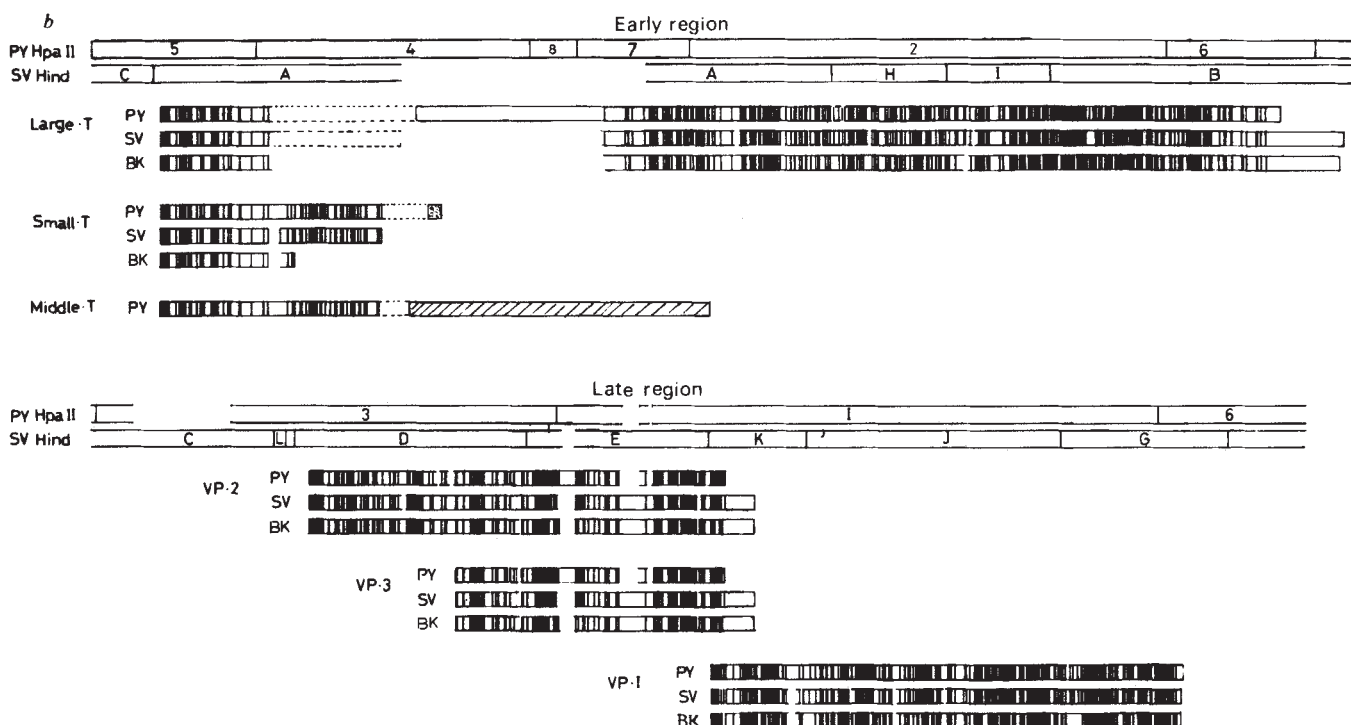


Fig. 4 *a*, A histogram which compares the homology in the genomes of polyoma virus and SV40, as derived from their DNA sequences (see Fig. 1 and refs 7 and 8). The polyoma virus and SV40 physical maps (Fig. 2a) are linearised about their respective origins of replication and divided into early and late regions. Gaps are left in the physical maps either where there is no corresponding sequence in the two genomes or where necessary in order to maximise homologies in adjoining regions. The location of the viral proteins relative to the genomes is shown. Percentage DNA homology, calculated for every 45 nucleotides, is plotted against sequence location in the genome. Homology becomes meaningful when it exceeds the 25% random expectation, and this is indicated by a solid line drawn across each figure. *b*, A comparison of the amino acid homology presumed to be present in individual proteins of polyoma virus and SV40. The polyoma virus and SV40 physical maps (see Fig. 2a) have been linearised about their respective origins of replication and divided into early and late regions, that is, regions coding for T antigens, and capsid proteins, respectively. From the DNA sequences (see Fig. 1 and refs 7 and 8), the amino acid compositions for each protein have been deduced and compared. A homologous amino acid occurring in the same protein among the two viruses is indicated by a black line (■). Thus, black areas represent homologies and colourless areas non-homologies. Gaps are left whenever necessary in order to maximise homology. Dotted lines indicate regions of the DNA which are noncoding for any part of a particular protein under consideration. In a similar fashion, polyoma virus has been compared with the predicted protein compositions of BK virus (MM strain) as deduced from its DNA sequence³⁷. The late region of SV40 has a number of duplicated sequences outside the coding region for which there is no counterpart in polyoma. This is indicated by a gap in the polyoma virus physical map. In the early region of polyoma virus DNA, there is a sequence which appears to have no counterpart in SV40. This is indicated in a similar manner by leaving a gap in the SV40 physical map. Neither SV40 nor BK virus appears to code for a protein which corresponds to the middle T antigen of polyoma virus, the body of which is encoded within the polyoma virus early sequence, indicated by cross-hatching (//). The assessment of per cent homology between viruses is subject to the uncertainties inherent in the alignment of the genomes. When polyoma virus and SV40 are aligned in the manner shown here, leaving occasional gaps but rearranging no sequences, genes coding for small t and large T antigens and the VP2 proteins had between 36 and 38% nucleotide homology, and the VP1 genes had about 53% homology (see *a*).



SV40, as shown in Fig. 4b. Similarity is also found in the overall organisation of the two early coding regions, as illustrated in Fig. 2a as well as in the regions immediately around their origins of replication¹¹. However, nowhere in the two genomes does the homology over any length observable by electron microscopy exceed 60–70%. This may explain why earlier hybridisation studies failed to reveal homologies between the two viral DNAs and only in exceptional conditions was any heteroduplex formation observed³⁹. In the latter case, the heteroduplex involved the DNA sequences which lie in a part of the polyoma virus and SV40 VP1 genes which code for the N-termini of the proteins and this is not even a region of exceptional sequence homology. Thus, earlier studies have probably not given a fair estimate of the similarities of the two viral genomes. This conclusion has been reached from studies on SV40 and its much closer relative, BK virus³⁶. However, recent hybridisation studies, in less stringent conditions than previously used, do reveal limited regions of homology between polyoma virus and SV40 DNAs (ref. 40). Sequencing the genomes of similar viruses thus emphasises how little is actually known about the chemistry behind heteroduplex formation, or that involved in liquid hybridisation studies, and presents a more accurate account of relationships between species.

The similarities between polyoma virus, SV40 and BK virus are very striking and if these viruses evolved from a common ancestor, it becomes apparent which portions of the genomes have been conserved during evolution just as data discussed below may indicate changes in the genome which occurred during evolution. The differences among the viruses are also very noticeable (see Fig. 4) and may ultimately be found to be of considerable biological importance. These differences appear to lie in three parts of the genome: at the end of the early and late regions, and in a part of the early coding region. Considering first the ends of the early and late regions, SV40 has a large (about 2% of the genome) noncoding region between the portion of its genes which code for the C-termini of large T antigen and VP1 (refs. 7 and 8) (see Fig. 2a). No such large coding gap is found in the polyoma virus. In the latter, sequences which code for the C-termini of large T antigen and VP1 are separated by only seven base pairs. Further, in the noncoding regions around the viral origins of replication of the viruses, although there is considerable homology in the small portion of the sequences which may define the initiation of replication^{11,41,42}, there appears to be little sequence homology in the rest of these regions. Note that for polyoma virus, the region under consi-

deration is somewhat less than 9% of the genome, whereas in SV40, it is about 13%. However, a search for homologies is somewhat hampered by the fact that much of the sequence in the noncoding part of SV40 consists of DNA repetitions^{7,8}; a large part of the region can be deleted without affecting the viability of the virus⁴³. It is not yet clear which sequence is redundant and which is essential in SV40. With polyoma virus, there appears to be much less, if any, analogous sequence redundancy. The noncoding regions around the origins of replication in the viruses may essentially be a control area of each virus. As such, it may be expected that the region might resemble a functionally related region in its host genome rather than another virus, even a related one, with a different host.

Finally, there also exists what currently appears to be the most important biological difference between the viruses, which is represented by the part of the early region in polyoma virus between about 86 and 99 units on the physical map. This region appears to code for the unique part of middle T antigen, as well as for part of large T antigen (refs 4 and 17). It seems that there is no comparable region in SV40 and without extensive sequence rearrangements, it is not clear how SV40 (or BK virus) could code for a protein comparable to the polyoma virus middle T antigen (see Figs 2 and 4). Studies with mutants indicate that this region in polyoma virus seems to be of critical importance in the virally induced transformation of cells and in tumour formation⁴. If polyoma virus has a gene which is responsible for the transformation of cells, and there is circumstantial though by no means conclusive evidence to suggest that it has, the middle T antigen is the best candidate for the product of this gene. If this proves to be the case and if SV40 does not code for a similar protein, then transformation may proceed by entirely different pathways, or involve different proteins, in the two viruses. An understanding of the differences between the two viral genomes may be essential in ultimately defining this property of the viruses. The known DNA sequences should now allow proper viral mutants to be constructed, by site-specific mutagenesis⁴⁴ or other procedures, which can establish the direct structural and functional relationships of these oncogenic viruses.

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Amino acid sequence changes in the haemagglutinin of A/Hong Kong (H3N2) influenza virus during the period 1968–77

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Haemagglutinin molecules from nine strains of A/Hong Kong/68 (H3N2) influenza virus, isolated between 1968 and 1977, were examined for changes in amino acid sequences. At least 18 changes, 9 of which were located precisely, occurred in the soluble tryptic peptides of the large haemagglutinin polypeptide (HA1) during this period. These peptides contained 262 residues (82% of HA1). In HA2, only two changes in 129 residues (58% of HA2) were detected. Sequential changes at a particular locus were not found; and as far as we can tell, once an amino acid changed, it did not change again in any subsequent variant examined.

INFLUENZA persists as an uncontrollable infection in man largely because of the high frequency with which antigenic changes occur in the surface proteins of the virus. Both influenza A and B viruses have two distinct surface antigens, the haemagglutinin (HA) and neuraminidase (NA). These may undergo a gradual change (antigenic drift) under increasing antibody prevalence in the population. Significant drift in the HA antigen is often associated with influenza epidemics of varying impact which occur without any regular time intervals. Occasionally, the HA and NA antigens of influenza A virus may abruptly undergo a complete change (antigenic shift) and bring about an influenza pandemic. Two recent influenza pandemics occurred, in 1957, when both the HA and the NA antigens changed ('Asian 'flu'), and in 1968, when only the HA antigen changed ('Hong Kong 'flu'). Influenza B viruses undergo antigenic drift, but are not known to undergo antigenic shift and have not been associated with pandemics.

Antigenic drift in type A influenza virus is believed to occur by the selection, in an immune population, of mutant virus particles with altered antigenic determinants on the HA and NA proteins. Peptide maps have shown that antigenic shift is associated with major differences in the amino acid sequence of the HA, and drift with minor differences¹.

Antigenic drift in the Hong Kong subtype

Since the appearance of the Hong Kong (H3N2) subtype of influenza type A virus in man in 1968, drift has occurred, resulting in the appearance of new variants, several of which are listed in Table 1. The original A/Hong Kong/68, and some of these variants, such as A/England/42/72, A/Port Chalmers/1/73, A/Victoria/1/75 and A/Texas/1/77, became widespread, causing epidemics in many countries, but most of the others were associated with only minor outbreaks and quickly disappeared². Antigenic relationships between the viruses listed in Table 1 have been described previously^{3,4}.

In this study, we have examined the HAs of these nine Hong Kong variants for amino acid sequence changes, by matching the compositions of their soluble tryptic peptides with the known amino acid sequence of the homologous peptides from the Hong Kong variant, A/Memphis/102/72 (ref. 5).

The HA monomer is coded by a single, monocistronic segment of RNA^{6,7} and is synthesised as a single polypeptide chain which is then normally cleaved by proteolytic enzymes to give two chains, HA1 and HA2, held together by disulphide bonds⁸. These two chains can be readily separated by column chroma-

tography or density gradient centrifugation after denaturation and disruption of the disulphide bonds. HA1 is the N-terminal portion of the HA⁹, has a molecular weight of 47,000 (ref. 10) and contains most of the carbohydrate^{10,11}. HA2 is the C-terminal portion of the HA⁹, has a molecular weight of approximately 30,000 (ref. 10), carries very little carbohydrate^{10,11} and contains the hydrophobic tail by which the HA is attached to the viral membrane^{9,12,13}.

Sequence changes in HA1

Maps of the soluble tryptic peptides from HA1 of A/Hong Kong/68 and A/Memphis/102/72 viruses showed several differences¹⁴. Therefore, a detailed study of the changes occurring in nine different Hong Kong variants (Table 1) was carried out.

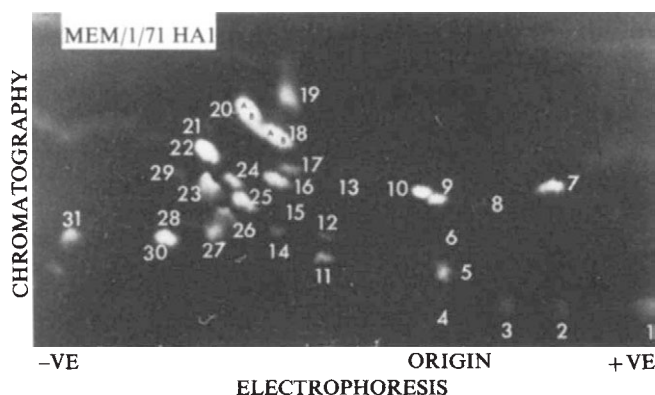


Fig. 1 Map of the soluble tryptic peptides from S-carboxymethylated HA1 of A/Memphis/1/71 influenza virus. Virus was grown in the allantoic sac of 11-d-old chick embryos and purified as described previously²². Haemagglutinin was isolated by electrophoresis of SDS-disrupted virus on cellulose acetate strips¹⁹. Heavy (HA1) and light (HA2) chains were separated by centrifugation on a guanidine hydrochloride–dithiothreitol density gradient⁸. The isolated chains were S-carboxymethylated as previously described²⁰, dialysed and precipitated with 3 volumes of ethanol. Digestion with trypsin (TPCK treated; Worthington), and mapping of the peptides soluble at pH 6.5 were as described elsewhere¹⁴. Peptides were located by staining with fluorescamine (10 µg per ml) in acetone containing 0.5% pyridine²¹, eluted from the maps with 6 M HCl, hydrolysed in sealed, evacuated tubes for 22 h at 105 °C and analysed on a Beckman 119CL amino acid analyser. Tryptophan was qualitatively determined by staining duplicate maps with Ehrlich reagent. These methods were followed to prepare maps of HA1 and HA2 from the H3N2 viruses listed in Table 1. These viruses were grown as recombinants with the neuraminidase from A/Bel/42 (HON1).

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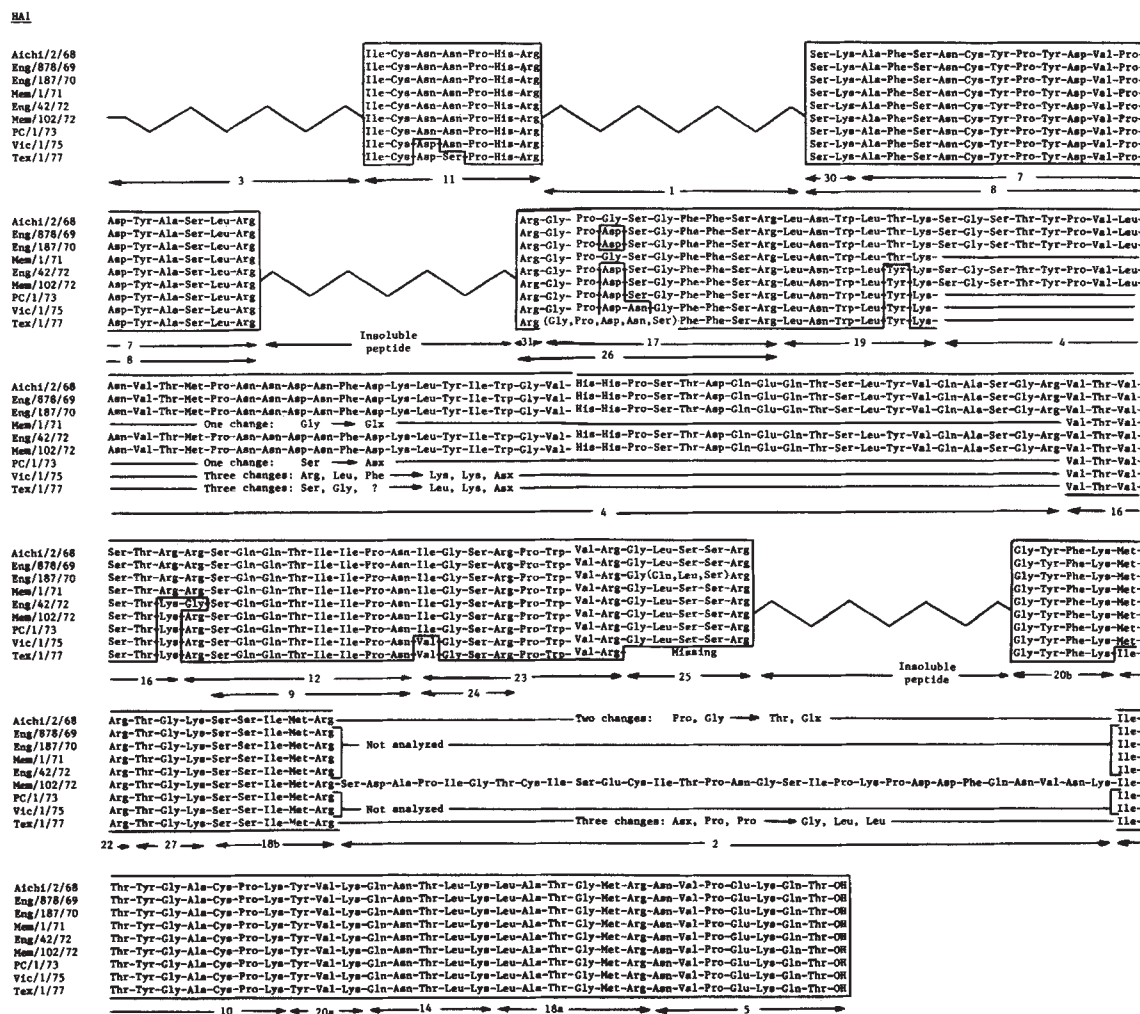


Fig. 2 Comparative partial amino acid sequences of the HA heavy chains (HA1) from nine different Hong Kong influenza A variants isolated between 1968 and 1977. The amino acid sequences were determined for the A/Memphis/102/72 strain (ref. 5 and C. W. W. and T. A. D., unpublished). Sequences for the other strains were deduced from the amino acid composition of their tryptic peptides. Boxed areas indicate regions of identical sequence and the residues which changed are indicated. It is not known which of the two Gly residues of Vic/75 peptide 17 changed to Ser in Texas/77, nor how one of the two Ser residues in Aichi/68 peptide 25 changed to Gln in Eng/187/70. Peptide numbers refer to the spots shown in Fig. 1. The four zig-zag lines denote regions of sequence associated either with the large peptides 1 and 3, which have not been fully sequenced and which therefore were not reliably analysed by single hydrolysis, or with two large insoluble tryptic peptides which we were unable to separate and analyse. The sequence of one of these (between peptides 25 and 20b) for the Mem/102/72 strain has been published⁵ but no sequence data for this peptide from the other strains are available. We have previously found that variants of A/Memphis/1/71 virus selected with monoclonal antibodies showed single amino acid sequence changes¹⁵. These were in peptide 11, where the Asn at position 4 changed to Lys; peptide 16, where the Ser changed to Tyr; peptide 17 (26) where the Pro changed to Ser, Leu, Thr or His, and peptide 25, which was missing in one monoclonal variant, although the change responsible was not determined.

Figure 1 shows a map of the soluble tryptic peptides from S-carboxymethylated HA1 of A/Memphis/1/71. The assumed sequences and locations of these A/Memphis/1/71 peptides, based on their amino acid composition¹⁵ and the available sequence data for the closely related A/Memphis/102/72 strain⁵, are shown in Fig. 2 with the corresponding data for the soluble HA1 tryptic peptides from the other eight Hong Kong influenza A variants examined.

Figure 2 shows that most of the primary structure of Hong Kong virus HA remained unchanged during the period 1968–77. In the soluble tryptic peptides, which contained 262 amino acids (82% of HA1), 18 changes in sequence could be detected. Of these, nine were located precisely, but in the four large peptides (1, 2, 3 and 4), where changes in composition were seen, we cannot know the exact number of sequence changes without more detailed analysis of sub-fragments. There may be more changes in sequence than detected by composition analysis. The regions of sequence not covered in this report are associated with two large insoluble tryptic peptides that will require additional enzymatic digestion before sequence analysis.

In most cases, once an amino acid residue had changed, it did not change again in any of the other variants subsequently

isolated. Exceptions to this were seen in four cases where apparent reversion to the original amino acid was seen. Thus, in peptide 12, the arginine in position 1 in A/Aichi/2/68, A/England/878/69, A/England/187/70 and A/Memphis/1/71 was replaced by glycine in A/England/42/72 but is arginine in the subsequent strains. A likely explanation is that this isolate of A/England/42/72 was not the virus that gave rise to the 1972–77 variants. As reported previously¹, peptide 25 from HA1 of A/Queensland/7/70 virus had the leucine residue replaced by glutamine. Although this change was not found in

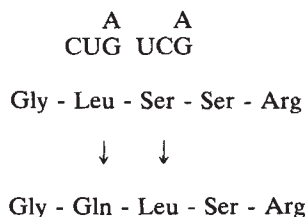
Table 1 Hong Kong (H3N2) variants examined

A/Aichi/2/68	A/Memphis/102/72
A/England/878/69	A/Port Chalmers/1/73
A/England/187/70	A/Victoria/1/75
A/Memphis/1/71	A/Texas/1/77
A/England/42/72	

All these viruses were obtained from WHO reference laboratories, with the exception of the Memphis strains, which were local isolates. The viruses were grown as recombinants with the neuraminidase from A/Bel/42 (HON1). The recombinants were cloned twice at limit dilution before analysis.

any of the Hong Kong strains examined in this report, the Eng/187/70 strain had a Ser → Gln change in the same peptide (see Fig. 2). The most likely explanation is that the A/Queensland/7/70 and Eng/187/70 strains did not persist in nature and did not give rise to subsequent strains. The third example is in peptide 4, where in Mem/1/71 there is a Gly → Gln change not seen subsequently. The fourth example of apparent reversion back to the original residue can be seen in peptides 17/26 where the glycine at position 4 is replaced by aspartic acid in the 1969 and 1970 England variants, but not in the 1971 American strain, A/Memphis/1/71. All subsequent variants (1972–77) have aspartic acid in this position. Although the nine variants studied here represent Hong Kong variants isolated in chronological order, they do not necessarily represent an exact genealogical series. Variants showing this Gly → Asp change in peptides 17/26 apparently arose first in England, then spread to, or arose independently in, the US.

Almost all the changes we have observed in compositions of small peptides can be accounted for by a single nucleotide change. One exception is in peptide 25 of Eng/187/70 (and no other strain we have examined), where there is a Ser → Gln change. It would be interesting to sequence this peptide, because the sequence could show two changes, for example,



The Leu → Gln change has been found in Qld/7/70, although peptide 25 in subsequent strains is the same as in 1968 and 1969 strains until Texas/1/77, where the peptide disappears (indicating some other change).

The other 2-base change appears in peptide 19 of Eng/42/72 and Mem/102/72, and persists through to Texas/1/77, being Thr → Tyr



In the large peptides 4 and 2, several of the observed changes in composition would require at least two nucleotide changes in the RNA.

Sequence changes in HA2

Figure 3 shows the map of the soluble tryptic peptides obtained from S-carboxymethylated HA2 of A/Memphis/1/71 virus. Using the sequence data available for A/Memphis/102/72 (ref. 5) it was possible to deduce the sequences of most of these A/Memphis/1/71 peptides from their amino acid compositions¹⁵. The location of each of these peptides in the partial sequence of HA2 is shown in Fig. 4. These peptides account for 129 residues (58% of the total HA2 sequence), the rest being insoluble and not examined.

Although we have not been able to characterise the exact changes, the data presented in Fig. 4 show that in the 20 soluble HA2 peptides examined, only two differences could be detected between the first and last Hong Kong strains. This suggested that extensive variation would not be found in the intermediate strains, and therefore these were not examined.

Relationship between sequence changes and antigenicity

The analysis of amino acid sequence changes in natural influenza variants may not necessarily indicate which portions of the sequences make up the antigenic determinants. Some of the

changes observed may be unrelated to the antigenic differences between the various HAs. Considering the large antigenic difference between Hong Kong/68 and Texas/77 viruses³, and the large number of antigenic sites on the HA molecule¹⁶, the number of sequence changes found associated with antigenic drift was surprisingly small. In most cases, only one or two amino acid changes were seen between neighbouring drift strains. The heavy chain (HA1) from the last member of the series, A/Texas/1/77, differed from that of the first, A/Aichi/2/68, by about 18 out of 262 residues, whereas the light chain (HA2) showed only two amino acid substitutions in the soluble tryptic peptides during antigenic drift in this period.

We do not know which of the changes in sequence found to occur between 1968 and 1977 were responsible for the changes in antigenicity which occurred in the Hong Kong virus during this period. In a previous study¹⁵, influenza variants of A/Memphis/1/71 were selected with monoclonal hybridoma antibodies and examined for changes in amino acid sequence and antigenicity. These variants showed dramatic changes in antigenicity, having completely lost the capacity to bind the monoclonal antibody used for their selection. These changes were found to be associated with a single amino acid substitution in HA1, which in every case required only a single base change in the RNA. No changes were seen in HA2. The HA1 tryptic peptides in which alterations occurred in the variants were the same as those in which changes occurred in the field strains, but in every instance the amino acid changes in the monoclonal variants were different from those found in the field strains (Fig. 2).

The recombinant viruses we used as the source of HA were cloned twice at limit dilution before analysis; this may have led to the separation of variant viruses with sequence changes in the HA. Furthermore, HA molecules of viruses isolated late in the Hong Kong era, antigenically related to the 'Victoria' and 'Texas' strains, may well have a number of sequence changes from the Victoria and Texas strains we examined. Asian (H2N2) viruses isolated in different parts of the world in 1968 at the end of the H2N2 period showed considerable differences in their HA1 and HA2 tryptic peptide maps¹⁷.

Electron microscopy of antibody molecules attached to the HA has shown that some (and possibly all) of the antigenic determinants are situated in a region just below the tip of the HA spike¹⁸. In the present work we have found changes along the whole length of the HA polypeptides, which may reflect the way in which the polypeptide chains are folded within the HA spike.

On the other hand, we do not know which of the sequence changes are responsible for the antigenic differences between the various viruses and which are not. Neither do we know if the sequence changes responsible for the antigenic changes occur in

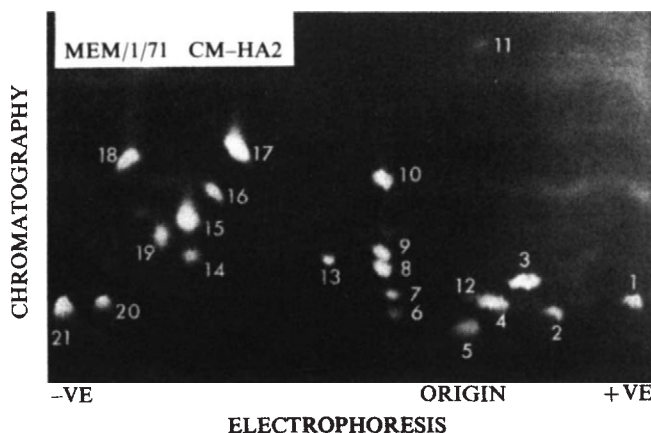


Fig. 3 Map of the soluble tryptic peptides from S-carboxymethylated HA2 of A/Memphis/1/71 influenza virus. The methods for virus growth, HA2 purification, enzyme digestion and peptide mapping are described in Fig. 1 legend.

HA 2

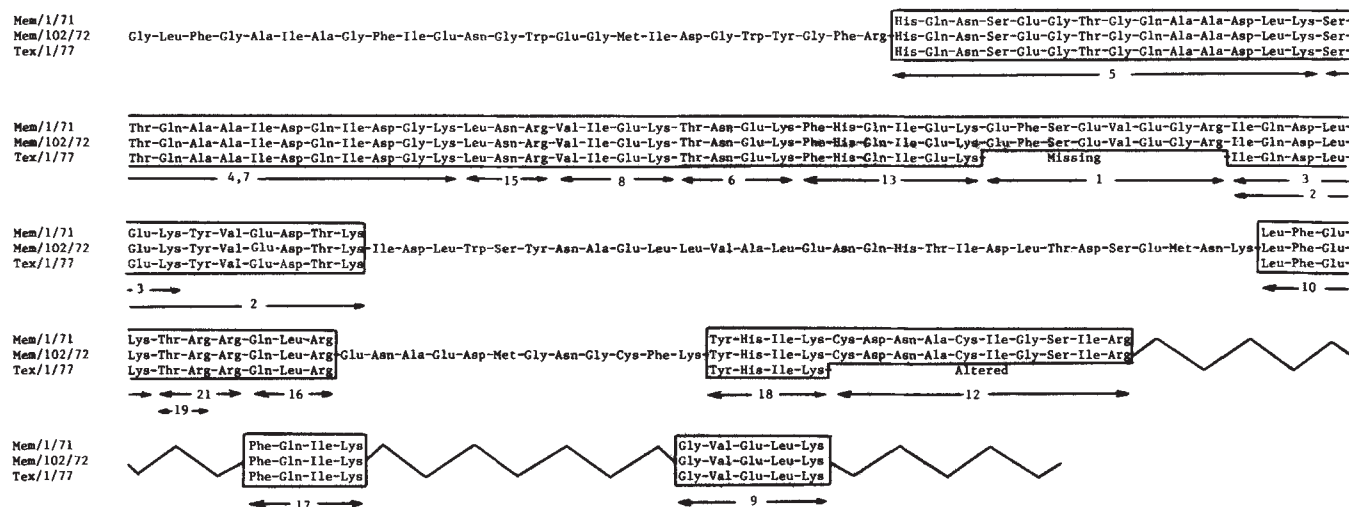


Fig. 4 Comparative amino acid sequences of A/Memphis/1/71, A/Memphis/102/72 and A/Texas/1/77 HA light chains (HA2). The amino acid sequences have been determined for the A/Memphis/102/72 strain⁵. Sequences for the other two strains were deduced from their amino acid compositions. The peptide numbers refer to the corresponding spots shown in Fig. 3. The regions of residues 1–25 and 89–117 and much of the C-terminal sequence involve large insoluble tryptic peptides not analysed in this study. In peptide 18 the sequence has been revised and corrected from that given previously⁵.

those amino acids which constitute the antigenic determinants, or whether the sequence changes occur elsewhere in the molecule and cause conformational changes which alter the antigenic determinants.

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LETTERS

IR sources in the Southern Coalsack

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A preliminary survey of four dark globules in the Southern Coalsack, an area of conspicuous obscuration in the southern sky, has revealed seven IR sources, which are most probably heavily obscured background stars. We report here that the wavelength dependence of extinction is not significantly different from that in the general interstellar medium. Spectroscopy of these stars, and of still more heavily obscured stars that should be revealed by a deeper survey, would permit detailed analysis of 'proto-protostellar' material. Although these are probably background stars, the numbers found in our small sample at least suggest association with the globules themselves; there is growing evidence for star formation within or associated with dense dark globules.

Bok¹ has reviewed the important role of dark globules in our understanding of star birth in the Galaxy, and has suggested the use of IR photometry to probe these heavily absorbing 'proto-protostars'. The Southern Coalsack, the finest dark nebula in the southern Milky Way, contains many globules, which appear as roundish, sharply bounded dark nebulae a few arc-min in diameter. Bok, Sim and Hawarden² have superimposed blue-green and far-red photographs of Globule 2 of the survey of Tapia³, the far-red images revealing peripheral reddened background stars invisible on the blue-green photographs.

We have used the 0.75 m telescope of the South African Astronomical Observatory to make a quick survey of four Coalsack globules, numbers 1, 2, 3 and 9 in Fig. 1 of ref. 2, at a wavelength of 2.2 μm (*K* magnitudes). The areas scanned were squares of 7 $\times$ 7 arc min in each case. For illustrations we again used two photographs taken with the SRC 1.2 m Schmidt telescope at Siding Spring, Australia. Their star images were almost superimposed in a modified Zeiss blink comparator, and Fig. 1 shows photographs of the four globules taken from the TV monitor. Black images correspond to stars in the far-red photograph ($\lambda \sim 0.8 \mu\text{m}$), and displaced white images to those in the blue-green photograph ($\lambda \sim 0.5 \mu\text{m}$); again, far-red images unaccompanied by blue-green images can be seen within the globule boundaries, representing heavily reddened background

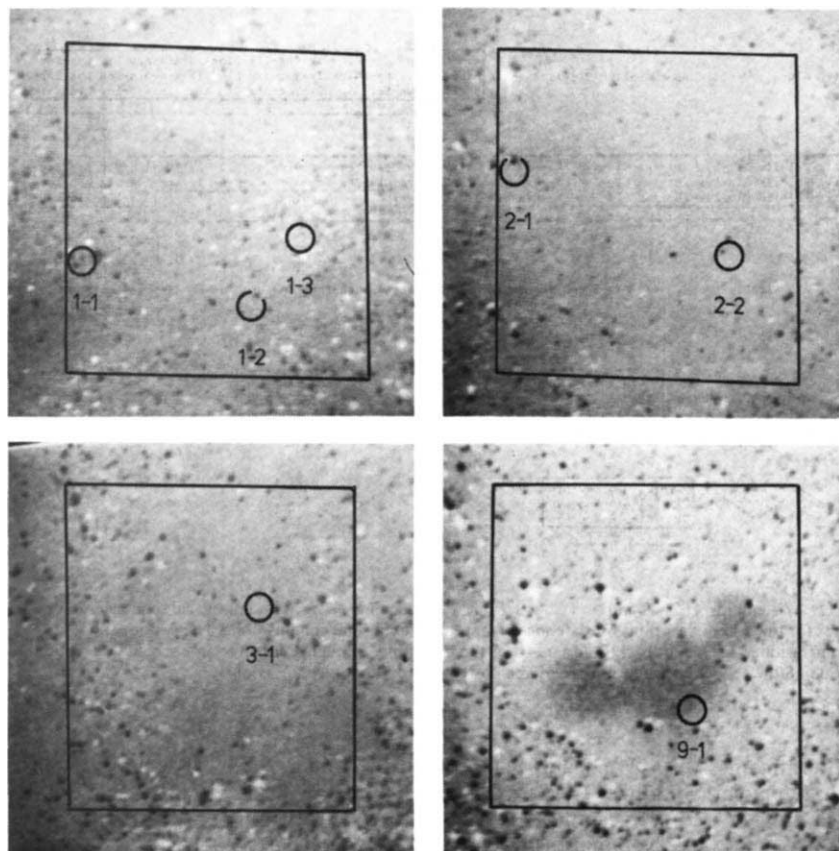


Fig. 1 TV monitor displays of the globules observed. Squares (7×7 arc min) show the areas scanned, and circles the locations of IR sources found. North is at the top, east to the left. Images from the far-red photograph are shown in black, those from the blue-green photograph in white.

stars. Boundaries of the square areas scanned are indicated, and circles enclose the locations of the brighter IR sources found, whose positions are listed in Table 1. In no case was any star visible in the photometer fields centred on the sources. The 3σ detection limit of the scans was $K \sim 8.5$. Several sources were detected, and results of subsequent J ($1.25 \mu\text{m}$), H ($1.6 \mu\text{m}$), K ($2.2 \mu\text{m}$), L ($3.4 \mu\text{m}$) photometry of the brighter ones are given in Table 2. Errors are of order 0.03 mag for J , H , K and 0.1 mag for L .

On the assumption that these are background stars whose light has been reddened by passage through the globules, we compare in Fig. 2 their ($J-H$, $H-K$) colours with those of an unreddened $M0$ star⁴. They lie on a locus close to that predicted by the normal interstellar extinction law (Table 12 in ref. 5). It is statistically most likely that random background stars are cool giants of spectral type K or M , but the assumption of early spectral type would force the extinction line through the origin and imply an abnormally steep slope. Elias⁶ also finds a normal extinction law in studies of extended dark cloud complexes.

To use the normal law to deredden the observed colours we require spectral types, giving intrinsic colours. In the absence of IR spectrophotometry we have estimated the probable range of total absorption on the basis of a range of spectral types from $G5$ to $M5$; the resulting ranges of total visual absorption A_V and intrinsic apparent magnitude V_0 are listed in Table 2. With the

exception of source 1-1 near the edge of a scanned area, the total absorptions almost certainly exceed 10 mag. Note that these heavily obscured sources were the brightest objects located in the scans at $2.2 \mu\text{m}$.

These quite large absorptions are found for stars that lie 1 or 2 arc min from the centres of the globules, where presumably the absorption is considerably higher. Bok¹ has conjectured that core absorptions at $1 \mu\text{m}$ do not exceed 7 mag, implying $A_V \leq 19$; our observations of peripheral IR sources suggest that higher central absorptions may occur. From star-counts, Bok *et al.*² estimated $A_V \sim 5$ in an outer annulus of Globule 2.

The ranges of deduced intrinsic magnitudes in Table 2, in conjunction with absolute magnitudes of giant stars from $G5$ to $M5$, imply distance moduli between 5.5 and 12, that is distances between 125 and 2,500 pc. The stars could thus lie within the

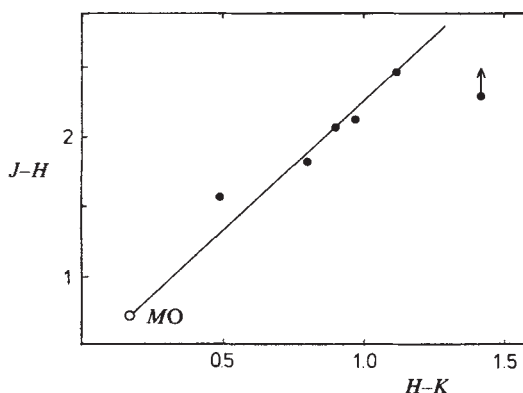


Fig. 2 IR colour indices $J-H$, $H-K$ for six of the heavily reddened sources, compared with those of a normal $M0$ star. The slope of the reddening line is 1.8, close to the normal interstellar value.

Table 1 Location of brighter IR sources

Source	α (1975)	δ (1975)
1-1	12 h 32 min 28 s	$-63^\circ 34'.4$
1-2	12 h 31 min 52 s	$-63^\circ 35'.3$
1-3	12 h 31 min 42 s	$-63^\circ 33'.8$
2-1	12 h 30 min 33 s	$-63^\circ 35'.7$
2-2	12 h 29 min 47 s	$-63^\circ 37'.4$
3-1	12 h 28 min 39 s	$-63^\circ 39'.1$
9-1	12 h 34 min 39 s	$-63^\circ 04'.2$

Table 2 Deduced intrinsic magnitudes

Source	<i>J</i>	<i>H</i>	<i>K</i>	<i>L</i>	<i>A_v</i>	<i>V₀</i>
1-1	8.23	6.65	6.16	5.94	6.5-9.5	7-12
1-2	9.72	7.58	6.61	6.03	13-16	7-11
1-3	10.03	8.20	7.40		10-13	7-11
2-1			8.60			
2-2	10.09	7.61	6.49	5.88	16-19	7-11
3-1	9.15	7.07	6.17	5.56	12-15	7-11
9-1		9.53	8.11			

globules (the estimated distance of the Coalsack is 175 pc) but more probably lie beyond them. On the other hand, the cumulative source-count model of Elias⁶ gives a probability of ~0.3 for finding a star intrinsically brighter than 7.5 (or $K < 8.5$ with $A_K = 1$) within one of the scanned areas, whereas in fact seven sources were found in four areas. It is thus tempting to suggest that the heavily reddened stars are physically associated with the globules. There is indeed increasing evidence for star formation associated with globules: Bok⁷ supports this view and gives an example of Herbig-Haro objects located within a dense globule. Our preliminary survey, in revealing the presence of intrinsically bright stars, and unexpectedly high absorption densities, again points in the direction of recent star formation in some globules.

We have surveyed only four of our Galaxy's population of dark globules, and cannot draw strong conclusions from this small sample. Evidently IR photometry should be used to probe a significant fraction of the observable globules. High-resolution

IR spectrometry should be attempted for the brighter sources located, to provide absorption spectra of proto-protostellar material.

For locating heavily reddened stars, array detectors working at shorter wavelengths may ultimately prove more powerful than single detectors scanning at, for example, 2.2 μm . At 1 μm the very large number of picture elements and high detection efficiency of a silicon CCD array will outweigh the lower globule absorption at 2.2 μm , except perhaps in the densest globule regions. R. Albrecht, B. J. Bok and G. Gozza (personal communication) have experimentally photographed Coalsack Globule 2 with such a device, and have indeed recorded stars close to the centre. But we have demonstrated that, until such devices become more widely available, scanning with an efficient InSb detector produces valuable results with a reasonable expenditure of telescope time.

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Coronal Faraday rotation during solar occultation of PSR0525 + 21

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The apparent position of the pulsar PSR0525 + 21 is within 1.3° (4.72 $R_\odot$) of the south solar pole on or about 13 June of each year. During these times of superior conjunction, the linearly polarised component of the pulsar radiation is subjected to an additional Faraday rotation due to the high electron density and strong magnetic field of the solar corona. We report here measurements of this coronal Faraday rotation in 1978 and 1979 at the 100-m radio telescope of the Max-Planck-Institut für Radioastronomie in Effelsberg which compared pulsar polarisation profiles at $\lambda = 17.4$ cm ($f = 1,720$ MHz) for times before, during and after occultation by the Sun. A significant residual rotation measure, which exceeded the interstellar value, was observed both years at times close to that of minimum solar approach.

Pulsars were recognised early as potential sources of probe signals for coronal investigations^{1,2}. Group delay time measurements, from which one may determine coronal dispersion measure (DM) by recording arrival times of pulses in well separated frequency bands, were first performed using the Crab Pulsar in 1969 at NRAO³ and Arecibo⁴. The additional delay in arrival time is proportional to the columnar electron content in the corona along the ray path pulsar/Earth, and is a very small fraction ($\approx 10^{-4}$) of the total delay due to interstellar dispersion. The Arecibo experiment was repeated in 1970, 1971 and 1973 to determine changes in the corona at later phases of

the solar cycle^{5,6}. Higher electron densities were measured at polar heliographic latitudes during solar maximum than during solar minimum.

In addition to measurements of coronal DM, a linearly polarised source could be used to determine the coronal rotation measure (RM) simply by tracking the source polarisation angle during occultation. The additional Faraday rotation of the plane of polarisation is proportional to the integrated product of electron density times the component of magnetic field along the line-of-sight in the corona. Values comparable with interstellar rotation measures can be achieved at solar elongations $\leq 2^\circ$. An experiment of this type was first attempted by Golnev *et al.*⁷ in 1962 using the Crab Nebula. Successive experiments on the same source were carried out by Sofue *et al.*⁸ and Berlin *et al.*⁹. Passive Faraday rotation experiments were also performed at low heliographic latitudes using the linearly polarised carrier signals of Pioneer 6 (ref. 10) and the two Helios spacecraft¹¹.

The first coronal Faraday rotation experiment using a pulsar as the source of the probe signal is described here. One decisive advantage of pulsars over continuously emitting sources is the built-in compensation for background noise (mainly from solar sidelobe interference), which can sometimes be several orders of magnitude more intense than the pulsar. The background is eliminated with a measurement during the 'off' phase of the pulsar period without having to drive the antenna off source. Preliminary measurements were taken in January 1978 when the pulsar PSR2045 - 16 came to within 5.6 $R_\odot$ of the Sun¹². The results of this first run were indicative, but not entirely convincing, that a coronal contribution was present.

Reliable measurements of coronal Faraday rotation were obtained, however, at the June occultations of PSR0525 + 21 in 1978-79. The occultation geometry for the two years is shown in Fig. 1. The apparent position of PSR0525 + 21 moves by the Sun from east to west at 1° per day in a plane parallel to the ecliptic. The UT start of days 11-17 June is marked by the series of large dots and the observation intervals are indicated by the heavy bars along the path. The paths of the intrinsically weaker pulsar PSR0540 + 23, which was used primarily as an ionospheric sounder in this experiment (see below), are also shown.

A stable integrated pulse profile of PSR0525+21 and two non-occulted 'control' pulsars (PSR0540+23 and PSR0823+26) was established using measurements for times well separated from the Sun. This profile, consisting of total intensity, linear intensity and polarisation angle at 256 equally spaced points across the pulse window, was termed the 'master' profile. During solar occultation the pulses were integrated just long enough to obtain what was felt to be a stable 'test' profile, which ideally differed from the master profile only in the absolute polarisation angle. This difference, after compensation for possible ionospheric effects, was attributed to the interposed solar corona. Only points within the test and master pulse windows with a high degree of linear polarisation were used in the determination of the mean weighted polarisation angle difference for a given test profile. The accuracy in determining this differential Faraday rotation is a function of integration time, solar offset (background noise) and intrinsic source intensity during the recording of each test profile. A typical error using this technique (one standard deviation of the weighted mean coronal rotation measure) was of the order of $\pm 1 \text{ rad m}^{-2}$.

When possible, the correction for the ionosphere was made using observations of PSR0540+23, whose ray path penetrates virtually the same ionospheric regions as that of PSR0525+21. It was originally thought that PSR0540+23 was not sufficiently intense for coronal sounding measurements. However, aside from the scintillation fading that occasionally degraded the signals of all pulsars, PSR0540+23 was determined *a posteriori* to be only slightly worse adapted for this experiment than PSR0525+21. The minimum offset distance for PSR0540+23 is the smallest of all known pulsars¹³, and is the only one available for coronal sounding at low heliographic latitudes.

The coronal rotation measure of the signal from PSR0525+21 on 12–15 June 1978–79 (ionospheric correction included) is presented in Fig. 2. One degree of Faraday rotation yields a rotation measure of 0.58 rad m^{-2} at the observing frequency of 1,720 MHz. The apparent distance of the pulsar from Sun centre (solar offset) is given for both years at the top of the figure.

The data for the two years are remarkably similar, particularly when one considers the adjustment for the two solar offset scales. A large negative Faraday rotation (mean coronal field directed away from observer), essentially symmetric about the point of closest approach, was recorded on both occasions. This coronal contribution first becomes large at solar offsets $< 5 R_{\odot}$, which is approximately the solar distance at which the solar wind undergoes a transition from a rapidly divergent flow (number

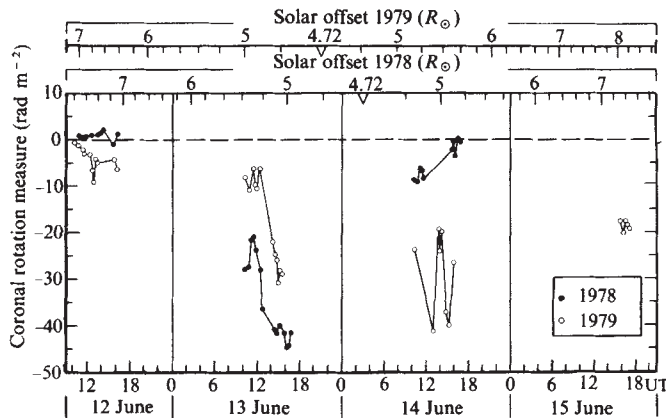


Fig. 2 Coronal rotation measure of the signal from PSR0525+21 during its solar occultations in 1978–79. The solid (open) points display the results obtained in June 1978 (1979). The minimum apparent solar offset of PSR0525+21 ($4.72 R_{\odot}$) is attained at a slightly different UT each year as shown in the two scales at the top.

density n goes as r^{-6} in semi-empirical models) to one which is purely radial ($n \sim r^{-2}$). This may help to explain the undramatic observations of PSR2045–16 in January 1978 (ref. 12), which did not approach the Sun closely enough. The magnitude of the coronal effect in Fig. 2 was comparable with that recorded in the ecliptic at the same solar offset even though the polar regions of the Sun are dominated by considerably less dense coronal holes^{10,11}.

The solar rotation measure is seen to be a continuous even function about the point of closest approach, so it may be assumed that the main contribution stems from a monopolar magnetic region situated over the south pole. This contrasts with previous spacecraft occultations in the ecliptic^{10,11}, where a field reversal about the mid-point of the occultation was observed (odd functions). As the south solar pole has a towards polarity in this phase of the present solar cycle, regions on the near side of the Sun (viewed from Earth) will cause the negative Faraday rotation that is observed. It is concluded therefore that either the polar hole region is greater in extent or contains higher electron density times magnetic field on the nearer Earth leg of the coronal ray path.

These observations have demonstrated that pulsars are the best natural sources available for the coronal Faraday rotation experiment. The highly polarised pulsed emission could be used for simultaneous recording of solar DM and RM, although no single ground facility is ideally instrumented for such observations. It is planned to coordinate a joint observational programme at Effelsberg and Arecibo for the 1980 tandem solar occultation of PSR0525+21 and PSR0540+23, which will occur during the Solar Maximum Year (SMY) and would thus profit from independent support not only from other ground-based observatories, but also from the Solar Maximum Mission (SMM), which will be in full operation by that time.

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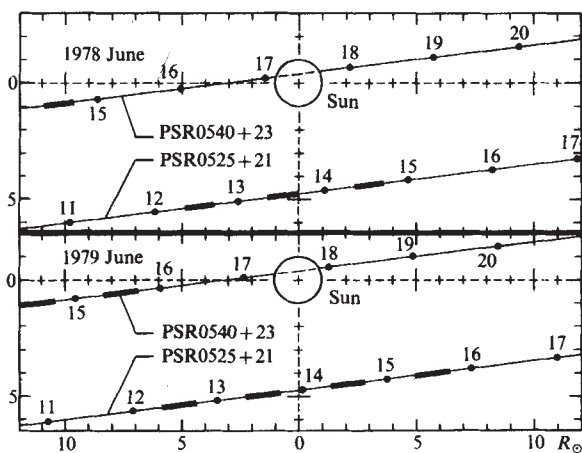


Fig. 1 Apparent paths of the pulsars PSR0525+21 and PSR0540+23 during their solar occultations in June 1978–79. The ray path pulsar/Earth moves parallel to the ecliptic from east to the west solar limb in this solar equatorial projection. The dots mark the start of a UT day, the associated number giving the June date for each year. The heavy bars along the path denote the observation intervals at the Effelsberg telescope.

Photo-sputtering of ice and hydrogen around Saturn's rings

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Rocketborne UV spectroscopic measurements have shown that a surprisingly dense cloud of atomic hydrogen surrounds Saturn^{1,2} which has been interpreted as being associated with the Saturn ring system. Verification of an UV emission cloud related to the rings is given by Pioneer 11 flyby measurements³. Several ring sources of atomic hydrogen have been proposed⁴⁻⁷, but abundance and lifetime estimates given below require a source which is much more potent than those previously considered. An alternate mechanism which satisfies this source requirement is proposed here, and involves photodissociation of surface water molecules on the icy ring material.

The rocket measurements of Weiser *et al.*^{1,2} show hydrogen Ly α emissions outside the disk of Saturn with a mean brightness of $200R \pm 50\%$. If this emission is produced by resonance scattering of the incident solar Ly α line with an efficiency⁸ of $2.3 \times 10^{-3} \text{ s}^{-1} \text{ atom}^{-1}$, then the total number of atoms within their viewing aperture is $N \approx 8 \times 10^{33}$ atoms (assuming optically thin conditions). The mean density is $n \approx 400 \text{ cm}^{-3}$. One can relate this abundance to a production rate through the hydrogen atom lifetime as discussed below.

Loss of H atoms can be caused by many processes, including photoionisation and charge exchange. However, the major loss of H atoms produced in, or near, the rings is through collisions and absorption by ring particles. Laboratory measurements⁹ of the reflection of H atoms by low temperature ice show reflection coefficients of 78%, that is, 22% of the collisions will result in loss of a hydrogen atom. The low density of the gaseous ring 'atmosphere' implies that molecular collisions are infrequent and that atoms follow keplerian orbits about Saturn. The trajectories of atoms created near the rings will pass through the rings generally once per orbit, twice if the orbital eccentricity is small. As the optical thickness of the rings is about unity¹⁰, then the probability of an atom striking a ring particle is $\approx (1 - e^{-1}) = 0.63$. For most of these collisions, the atom will be reflected, but roughly one impact in five will result in loss of an atom. For a nominal keplerian orbital period of $T = 4 \times 10^4 \text{ s}$, the resulting lifetime is $\tau = T/(0.63 \times 0.22) = 3 \times 10^5 \text{ s}$. With this lifetime, the source rate Q required to satisfy the observations is estimated to be $Q = N/\tau = 8 \times 10^{33}/3 \times 10^5 = 3 \times 10^{28} \text{ atom s}^{-1}$. Note that this derived source rate is insensitive to the actual trajectories. If the orbits extend beyond the field-of-view of the rocket aperture (which was used for the abundance estimate) then the fraction of time that an atom spends within the field is less than unity, and the orbital period T is also greater. As both the total abundance and lifetime estimates are increased, then the derived source rate is approximately the same.

The first investigations of H atom production by the rings were presented by Blamont⁴ and Dennefeld⁵, who considered

several sources, including sublimation and dissociation of ring material, and bombardment by meteoroids, the solar wind, and interstellar gas. Their calculated source rates are summarised in Table 1 and are evidently too small to account for the required value of $3 \times 10^{28} \text{ atom s}^{-1}$. Cheng and Lanzerotti⁶ proposed sputtering by high energy ions as a ring erosion mechanism. Assuming that peak jovian fluxes can be used to describe the saturnian magnetosphere, they predicted a hydrogen atom source rate $\approx 1.2 \times 10^{27} \text{ s}^{-1}$. However, the Pioneer 11 flyby has shown that the Saturn magnetosphere is much less intense than that of Jupiter, and the peak fluxes used by Cheng and Lanzerotti should be reduced by a factor of at least 20, and probably more, based on measured saturnian proton and electron fluxes^{11,12}. Furthermore, proton fluxes¹² in the vicinity of the rings are $< 10^{-2}$ of the peak values, which results in an ion-sputtering rate of $< 6 \times 10^{23} \text{ atom s}^{-1}$. An additional mechanism has been proposed by Ip⁷—neutralisation by ring particles of the photoelectron-ion flux flowing out from the Saturn ionosphere along magnetic field lines. The magnitude of this source is also insufficient to account for the observations (see Table 1).

Harrison and Schoen¹³ pointed out that UV radiation ($1,000 \leq \lambda \leq 1,800 \text{ \AA}$) will dissociate water molecules on ice surfaces and emit hydrogen atoms and hydroxyl radicals. Similar processes have been found to occur on UV irradiation of glasses and semiconductors^{14,15}. For photo-absorption which occurs in the outer few molecular layers of an ice crystal, the probability of escape from the crystal is high. At the orbit of Saturn, the molecular photodissociation rate¹⁶ due to solar UV radiation is $1.1 \times 10^{-7} \text{ s}^{-1} \text{ molecule}^{-1}$. If we assume that only the single outermost monolayer of molecules ($1 \times 10^{15} \text{ molecule cm}^{-2}$) of the icy ring material will contribute to the flux escaping from the solid, then we find a hydrogen atom flux of $1.1 \times 10^8 \text{ atom cm}^{-2} \text{ s}^{-1}$. Although the initial energy distribution cannot be accurately determined, most of these atoms should possess less than the 3 eV of energy required for escape from the Saturn system, due to simultaneous vibrational and electronic excitation of OH, collisions with other surface molecules, and subsequent collisions with ring particles.

The projected area of the A + B rings at the time of the rocket measurements was $1.4 \times 10^{20} \text{ cm}^2$, resulting in a total source rate of $Q = 1.5 \times 10^{28} \text{ atom s}^{-1}$. This value is compared with other mechanisms in Table 1.

The source rate for the mechanism proposed here is within a factor of two of the required value and is within the experimental error of the measurement. Considering the spirit of the calculations, the agreement is quite satisfactory, arguing that the proposed mechanism is a viable explanation for the observed Ly α glow. It may be possible to test this, and other, source hypotheses through the recent Pioneer 11 and forthcoming Voyager flybys. For the present mechanism, the source strength during these flybys will be smaller due to the smaller inclination of the ring plane relative to the Sun.

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Table 1 Ring H atom production rates

Mechanism	$Q \text{ (atom s}^{-1}\text{)}$
Photo-sputtering	1.5×10^{28}
Photoelectron-ion neutralisation	$9 \times 10^{23} - 9 \times 10^{24}$
Sublimation, dissociation	2.2×10^{24}
Meteoritic bombardment	1.8×10^{24}
Magnetospheric ion sputtering	$< 6 \times 10^{23}$
Solar wind	1×10^{23}
Interstellar gas	2.2×10^{22}

Cooling-rate dependence of thermoremanent magnetisation

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In using the thermoremanent magnetisation (TRM) of material heated in antiquity (usually pottery, bricks, stones or lava) to evaluate the magnitude of the geomagnetic field at the time of heating, little attention (apart from an observation by Thellier¹) has been given to the possibility that results are affected by the rate at which the sample is cooling while the TRM is being acquired. We report here an experimental observation of the effect in archaeological baked clay; it parallels the theoretical prediction of Dodson and McClelland-Brown² in respect of geological material.

The effect was first noted during a calibration experiment in which samples of Peruvian pottery³ were refired to 720 °C in a known laboratory field. The TRM acquired during the 7-h cooling from this refiring was then analysed routinely using the zero-field variant of the Thellier⁴ method. In this the cooling-times were of the order of 30 min. The samples were in the form of disks, (diameter 25 mm; thickness 8 mm) and before the experiment they had been thermally stabilised by prolonged baking. Magnetisations emplaced during the analysis were in the same direction with respect to the sample as the magnetisation being analysed; hence any effects due to sample shape or fabric anisotropy⁵ were avoided. The samples were measured on a Digico fluxgate archaeomagnetic-head magnetometer.

The results are given in Table 1, for both the Peruvian pottery and for samples fabricated from Oxfordshire clay. The field magnitude evaluated by the Thellier analysis is higher than the known field in which the samples had cooled during acquisition of the TRM being analysed, on average by 6% and 12% respectively. The values for the known field were obtained with a fluxgate magnetometer which had been calibrated against a proton magnetometer. The fields used in the course of the analysis were within 0.5% of 48.3 μ T.

Table 1 Evaluation by TRM of field intensity used for refiring

Specimen no.	Specific magnetisation ($\times 10^{-3}$ A m ² kg ⁻¹)	F ^{A*} (μ T)
P12P01P	2.5	52 $\pm$ 1
Q	2.4	53 $\pm$ 1
Y	2.5	54 $\pm$ 2
Z	2.5	54 $\pm$ 1
P43C01T	4.6	51 $\pm$ 1
U	3.3	55 $\pm$ 1
Y	4.8	50 $\pm$ 3
Z	3.7	55 $\pm$ 1
Islip 02	4.3	54 $\pm$ 2
03	5.5	54 $\pm$ 1
04	3.4	53 $\pm$ 1

The cooling time after refiring was 7 h. The cooling times during TRM analysis were ~30 min. For the Peruvian sherds, P12P01 and P43C01, the field intensity used for refiring was 50 μ T; for the Islip specimens it was within 0.2 μ T of 48.3 μ T. F^A is the value deduced for the refiring using Thellier zero-field analysis of the TRM acquired during cooling down from refiring.

* ± 1 s.d.

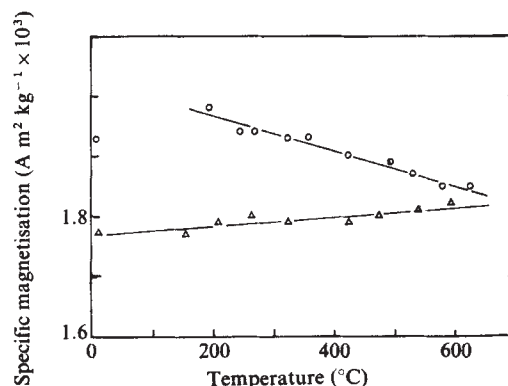


Fig. 1 Remagnetisation of Peruvian pottery sample (P12P10Y) following refiring to 720 °C. The TRM was measured (at room temperature) after remagnetisation during cooling from various reheating temperatures. Two sets of remagnetisations are shown: the first (○) followed a refiring in which the cooling time was 7 h, the second (△) followed a refiring in which the cooling time was half an hour. The latter was also the maximum cooling time for the remagnetisations. The magnetic field was 48 μ T in all cases.

In the above analytical procedure, at each temperature step the sample cools in zero field before being remagnetised. To avoid possible interference by a memory effect the following more direct experiment was carried out. After refiring to 720 °C, either with a cooling time of 7 h or of 30 min, the sample was reheated to successively increasing temperatures and cooled rapidly, in ~30 min, the reheating and cooling being in the same field as for the 720 °C refiring. After each step the magnetisation of the sample was measured (at room temperature); a typical result is shown in Fig. 1. Whereas for the 30-min cooling the sample shows a slight overall increase, by <1%, as the reheating steps proceed; for the 7-h cooling there is a decrease, by ~8%.

Figure 2 shows the results of a similar experiment carried out using the SQUID magnetometer (and its associated oven) developed for archaeomagnetic work by Walton⁶. In this the sample (a small 3-mm diameter by 3-mm thick core of Greek pottery) does not have to be demounted from its quartz presentation rod between in-field heating and measurement (at room temperature). After rapid cooling from 600 °C, in a few minutes,

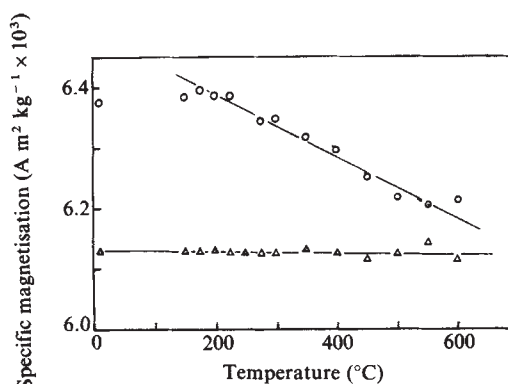


Fig. 2 Remagnetisation of Mycenaean pottery sample (1632a6) following refiring to 600 °C. The TRM was measured (at room temperature) after remagnetisation on cooling from various reheating temperatures. Two sets of remagnetisations are shown: the first (○) followed a refiring in which the cooling time was 2.5 h, the second (△) followed a refiring in which the cooling time was a few minutes. The latter was also the maximum cooling time for the remagnetisations. The magnetic field was 74 μ T in all cases.

Table 2 Effect of different cooling times on TRM evaluation of field intensity used for refiring

Sample reference	Overestimate relative to 5-min cooling times	
	For 2-h (%) cooling time	For 16-h cooling time
32a6.1 (Attic)	2	7
35a1.1 (Late Minoan)	7	11
P18 L03 (Peruvian)	9	14

TRM evaluation was by means of the Thellier method, using a SQUID magnetometer. Refiring was done in a small non-inductively wound oven external to the magnetometer which was lowered over the sample. For the 5-min cooling time the oven was lifted off the sample; for the 2-h cooling time it was left in position with the heater current switched off; for the 16-h cooling time the heater current was turned down at a uniform rate (by means of a motor driven Variac). For each sample a 5-min cooling time run was done initially and finally. The TRM evaluations from these pairs agreed to within $\pm 1\%$.

reheating and cooling (also in a few minutes) in the same field produced no change in magnetisation as successively increasing temperatures were used for the reheating. On the other hand, when the sample was cooled slowly from 600 °C, in 2.5 h, the subsequent magnetisation was 5% higher than for quick cooling, and as the reheating steps proceeded (each followed by quick cooling) the excess disappeared. For the slow cooling the heater current for the oven was turned down manually. The oven was non-inductively wound and the a.c. field at the sample site was $<20 \mu\text{T}$. In the large-sample experiments there was no current in the oven for the slow cooling, the fast cooling being achieved by blowing air on the samples after lifting off the oven.

The effect has also been observed⁷ with other samples (see Table 2); in all cases the TRM acquired for slow cooling is greater than for fast cooling. This is as Dodson and McClelland-Brown² predicted in considering samples in which the magnetic carriers are monodomain grains of haematite and magnetite. The basis of their prediction is that for a given group of grains the effective blocking temperature is lower in the case of slow cooling and therefore the fractional alignment that is blocked-in is higher, both because of the reduced thermal agitation at the lower temperature and because of the intrinsic increase of activation energy. Dodson and McClelland-Brown² consider very slow, geologically-relevant, cooling rates, in the range 6°C min^{-1} to 3°C Myr^{-1} ; they predict a 7% increase in TRM for an order of magnitude decrease in cooling rate. Our results accord with this prediction. The magnitude of the effect will depend on the magnetic mineralogy of the sample and hence it is not to be expected that it will be quantitatively the same for all samples. This aspect is under investigation.

In general, archaeological pottery and bricks fired in kilns will have cooled down after a couple of days⁸. The cooling times used routinely in this Laboratory for Thellier analysis are either 30 min or a few minutes. Hence the magnitude of the ancient field so evaluated may be larger than the true value by 10–20%; for laboratories employing longer cooling times, or for samples that cooled quickly after the archaeological firing (for example, in an open hearth or in a clamp kiln), the effect will be less. Clearly it would be desirable to investigate the strength of the effect in each type of sample and to make an appropriate correction, or to use laboratory cooling times of the same order as the presumed archaeological cooling time.

The significance for existing determinations of ancient intensity is that they are also liable to be overestimates. This will be significant when calculating the global radiocarbon content. If, for instance, the magnitude of the geomagnetic moment was in general 10% less than indicated by the global curve of Cox⁹, the historical radiocarbon production rate will have been $\sim 5\%$ more than currently assumed.

We thank Dr N. Gunn for help in the initial calibration experiment reported in Table 1 while he was at the University of Liverpool Department of Geophysics, also Dr M. Dodson for helpful discussion, and Professor R. Wilson for drawing to our attention the observations in ref. 1. We thank the NERC for a research grant, and a studentship to J.M.W.F.

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Bubbles and breaking waves

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The physical processes which control the transfer of gases between the atmosphere and oceans or lakes are poorly understood. Clouds of micro-bubbles have been detected below the surface of Loch Ness when the wind is strong enough to cause the waves to break. The rate of transfer of gas into solution from these bubbles is estimated to be significant if repeated on a global scale. We present here further evidence that the bubbles are caused by breaking waves, and discuss the relationship between the mean frequency of wave breaking at a fixed point and the average distance between breaking waves, as might be estimated from an aerial photograph.

It has been suggested¹ that there may be a causal relationship between whitecaps, or breaking waves, on the surface of a freshwater lake and the individual clouds or bubbles which have been detected acoustically below the surface. The clouds appear with a typical frequency of about 1 per min in wind speeds of 10 m s^{-1} and penetrate to depths of at least 5–10 m. To investigate the relationship, and other phenomena, we measured breaking waves in Loch Ness using a capacitance wire probe² and time-lapse photography.

The capacitance wire probe was placed on a mooring connected to shore³ in a water depth of $\sim 160 \text{ m}$ and 250 m from the shore, where the fetch for north-east or south-west winds is some 10 km. The wind speed was measured by an anemometer on a tripod in the loch. As the slope of the water surface in a breaking wave is large, the electronic circuit used with the probe was designed to detect large gradients in surface height. The signal voltage from the probe, proportional to the wave height, was passed through a differentiator and the output compared with a reference level which could be set according to the wave conditions. When the differentiated signal exceeded the reference, a spike generator was triggered which marked the record.

The slope, $\partial h/\partial x$, of the water surface is related to the time derivative, $\partial h/\partial t$, of the wave height measured at a point, by

$$\frac{\partial h}{\partial t} = c \frac{\partial h}{\partial x} \quad (1)$$

where c is the phase speed of the waves. For small deep-water waves $c = c_0 = gT/2\pi$, where T is the wave period and g the acceleration due to gravity⁴. For large amplitude waves the

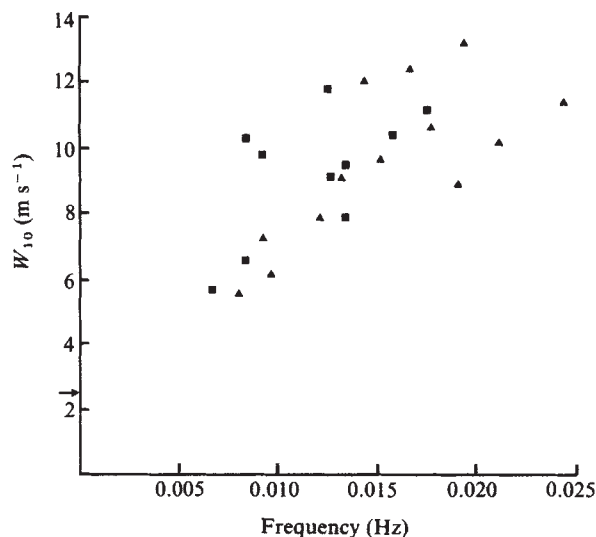


Fig. 1 Wind speed, W_{10} (corrected to 10 m) versus frequency f , of bubble clouds seen on a sonar record¹ (■), or frequency of breaking waves (▲), only one wave being counted when neighbouring waves break as the sonar record cannot resolve a time scale corresponding to individual waves, the bubble clouds from neighbouring waves are expected to merge. Observation periods of at least 20 min were used. The mean standard deviation of the number of waves in a five minute period is ~ 2.6 . The arrow marks the approximate wind speed at which whitecaps and bubbles are first observed.

phase speed is a function of the wave slope. The maximum slope of a regular progressive wave train in deep water⁵ is close to 30° when the phase speed⁶, $c = 1.0923c_0$, and hence the maximum value of the time derivative is approximately

$$\frac{\partial h}{\partial t} = 0.1004gT \quad (2)$$

(The greatest wave speed is estimated⁶ at $c = 1.09295c_0$ and hence equation (1), with $c = c_0$, may be used with an error of $<10\%$).

The capacitance wire probe was used to measure the mean wave period, T , estimated from the zero-crossing interval, and equation (2) used to estimate $\partial h/\partial t$ for a limiting wave. In practice the reference level setting was set empirically to trigger 'spikes' on an ink-plotting pen when a breaking wave could be seen at the probe, and considerably smaller values than the theoretical equation (2) were found to be suitable. Trouble was encountered by spikes being triggered near wave nodes where the wave slope is large but where no breaking or bubble entrainment occurs. Waves tend to break near their crests and these spurious spikes were avoided by incorporating a circuit which passed the signal spike only if the wave height exceeded a certain level, which was set in relation to the observed height of the existing wave field.

The success of the system in identifying breaking waves was tested by taking cine film of the probe and water surface from shore and by comparing the record of wave height and spikes with the photographic record. In calm weather tests were also made by artificially creating breaking waves in the wake of a boat which passed close by the probe. The detection system was insensitive to brief spikes of the order of 25 ms or less in which $\partial h/\partial t$ just exceeds the threshold. Waves less than the probe dimensions, ~ 10 cm, were not resolved either by the probe or by the camera, but larger waves with crest lengths exceeding this dimension were detected.

For wind-waves Loch Ness is effectively an enclosed basin, and swell from distant storms which might, at sea, induce wave breaking⁷, is absent. A relationship might therefore be expected

between the frequency of wave breaking and the local wind speed. Figure 1 shows the average frequency of wave-breaking events and individual bubble clouds seen in the sonar records¹, plotted against wind speed. There is considerable scatter, arising partly from the uncertainty in identifying individual bubble clouds in the sonar records and partly from the unsteadiness of the wind. There is an upward trend in both sets of points, commensurate with a zero value at a wind speed of $\sim 2.5 \text{ m s}^{-1}$ when whitecaps and bubble clouds are first observed. Whilst the frequencies are similar, the number of wave-breaking events exceeds that of the bubble clouds. A contributing factor may be that some waves, breaking 'gently', do not produce bubbles which penetrate below the depth of the pre-existing cloud.

The average period between the occurrence of breaking waves at the probe was compared with the mean distance between breaking waves approaching the probe. The latter was derived from time-lapse photographs of the water surface taken from the hillside above the loch. The width of the camera frame was established by taking photographs of a rubber boat of known dimensions. The time interval between frames was such that groups of waves would travel a distance well exceeding the frame width during the time interval, and thus be filmed only once. With the assumption that their statistics were uniform, the mean distance between whitecaps, $\bar{\lambda}$, was found by dividing the number of breaking waves observed to intersect a line along the loch axis (approximately the mean direction of wave advance) into the total effective frame length.

The large and steep waves which break, forming whitecaps, occur in groups. It was very rare to see two neighbouring waves break simultaneously and when this happened only one wave was included in the count. Figure 2 shows estimates of $\lambda/\bar{\lambda}$, where λ is the mean wavelength estimated from using the relation⁴ $\lambda = gT^2/2\pi$. Also shown are estimates of $\bar{t}/T$, where $\bar{t}$ is the average time interval, measured by the capacitance wire probe, between successive groups which contained breaking waves. About 58% of the groups which passed the probe with breaking waves contained more than one (none more than three). There is again much scatter in the observations (again variation in wind conditions may have contributed) but evidence of an increase in both $\lambda/\bar{\lambda}$ and $T/\bar{t}$ with wind speed. This is partly due to an increase in T (and hence λ) with wind speed from an average of 2.58 s at wind speeds of 6.17 m s^{-1} to 3.05 s at winds of 12.4 m s^{-1} . On average the values of $\lambda/\bar{\lambda}$ are significantly greater than those of $T/\bar{t}$ at the same wind speeds. (Mean values are 0.076 and 0.056 respectively at a mean wind speed of 9.9 m s^{-1}).

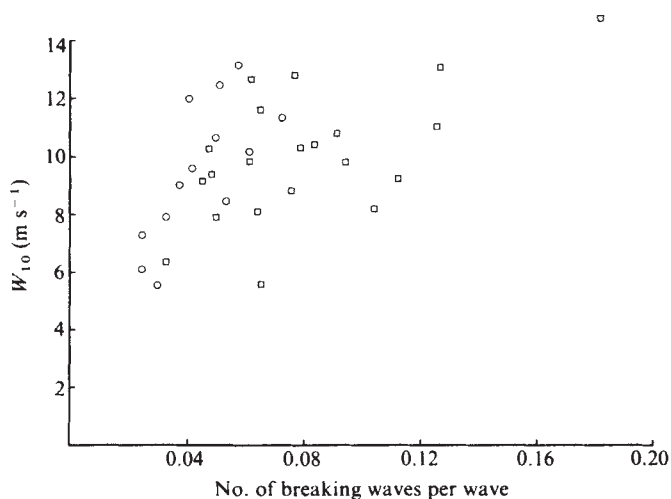


Fig. 2 Wind speed, W_{10} (corrected to 10 m) versus ○, $T/\bar{t}$ or □, $\lambda/\bar{\lambda}$, the number of breaking waves per wave, groups counting once only (see text). $\lambda/\bar{\lambda}$ was calculated over an effective horizontal length of at least 2,280 m.

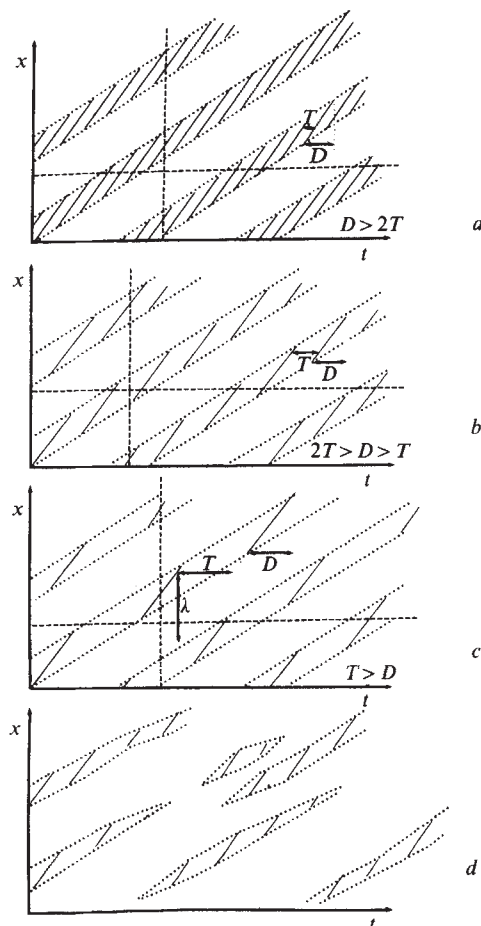


Fig. 3 Space (x)-time (t) diagram of the movement of breaking waves and groups. The heavy lines are breaking waves and the dotted lines are groups. Idealised diagrams when: *a*, $D > 2T$; *b*, $2T > D > T$; *c*, $T > D$; *d* is a sketch of a more realistic situation in which the groups break intermittently. The groups of breaking waves have been drawn closer together than they are in reality. The dashed lines x , constant; and t , constant indicate the way in which the breaking waves are sampled by the probe and by photographs respectively. In *a* these lines cut at least one breaking wave in each group. In *b*, t , constant misses, but x , constant always intersects, one or two of the breaking wave tracks, whilst in *c* both lines occasionally miss and more than one breaking wave in the same group is never sampled.

The regions of large waves, and hence whitecaps, advance at the group velocity⁸, which, in deep water, is half the phase speed of the waves. Figure 3*a,b,c* shows the waves in space-time coordinates. The individual breaking waves, advancing at the phase speed, are shown as solid lines of slope $c = \lambda/T$. The groups containing breaking waves advance at the group velocity and are indicated by a succession of these lines, forming a patch outlined by dotted lines with slope $c/2$. If the duration of persistence of a whitecap, D , is more than double the wave period (Fig. 3*a*) each group with breaking waves will contain at least one breaking wave when it is observed at constant time (by a photograph) or as it passes a constant position (the wave probe). If only one breaking wave in each group is counted, the ratio r of the mean distance between such successive observations at constant time and their mean period will be the group velocity, $c/2$. Hence the ratio

$$\chi = (\lambda/\bar{x})/(T/\bar{t}) = \frac{c}{r} = 2 \quad (3)$$

If, however, $2T > D > T$ (Fig. 3*b*), whilst each group containing breakers will be recorded by at least one breaking wave at the

probe (constant x), there are times when no waves at all will be breaking in a particular group, and hence $\chi < 2$. Moreover at most one wave at a time will be breaking in a group. If, as is often observed, $D < T$, then groups containing breaking waves may pass the probe with no breaking event registering. Groups containing multiple breaking waves are more likely to be recorded at a fixed location than to be seen in a photograph, as is indeed observed. The occurrence of multiple breaking waves at the probe suggests that D frequently exceeds T , whilst their absence in photographs implies that D is usually less than $2T$.

The observed mean value, $\chi = 1.65$, is consistent with this argument. Because the breaking waves are unlike the idealised model (but resemble Fig. 3*d*) there seems at present no obvious way in which χ may be related to the simple statistics of the wave field. A working value of χ would, however, be useful as it would then be possible, by finding $\bar{x}$ and T (or λ)⁸ from aerial photographs, to estimate $\bar{t}$ and hence to determine how often a stationary structure, for example, an oil rig or wave power device, would be subject to groups of breaking waves.

Whilst there is at least circumstantial evidence for a causal relationship between breaking waves and bubbles, more research is needed. Progress might be made by using narrow-beam upward-pointing sonar which could resolve details of the wave surface during wave breaking.

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Gallium porphyrins in bituminous coal

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Metalloporphyrins are minor constituents in crude oil and its relatives¹ but their presence in coal is not clearly established. This is surprising as such complexes are thought to arise from chlorophyll, and coal is largely of plant origin. Although coal samples were among the earliest examined in this context^{2,3}, most later workers^{1,4} followed Treibs⁵ in mainly considering crude oils, asphaltenes and shales. Weigelt and Noack² presented early evidence for chlorophyll derivatives in fossilised leaves from brown coal, while Treibs³ found trace amounts of metal-free porphyrins in various coals. Uspenskii and Gorskaja⁶ confirmed the latter finding, but observed that the porphyrin content of sapropelic coals such as cannel coal was much higher than that of hard coals. Metalloporphyrins in fossil plant material are important because they provide information on the geochemical transformations of chlorophyll. Moreover, metal complexes with organic ligands (such as the metalloporphyrins) are of particular interest in contemporary coal chemistry because they are probably extracted by the organic solvent in the conditions used in certain coal liquefaction processes, and hence may modify the activity of catalysts used in subsequent chemical reactions with the coal extract. We report here the isolation of a metalloporphyrin fraction from a British bituminous coal, and show that the principal metal present in the isolated material is gallium.

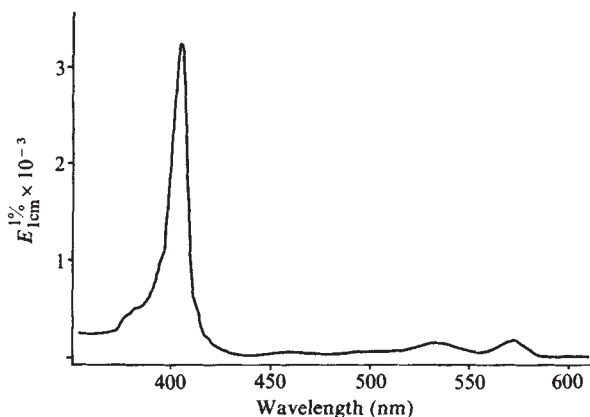
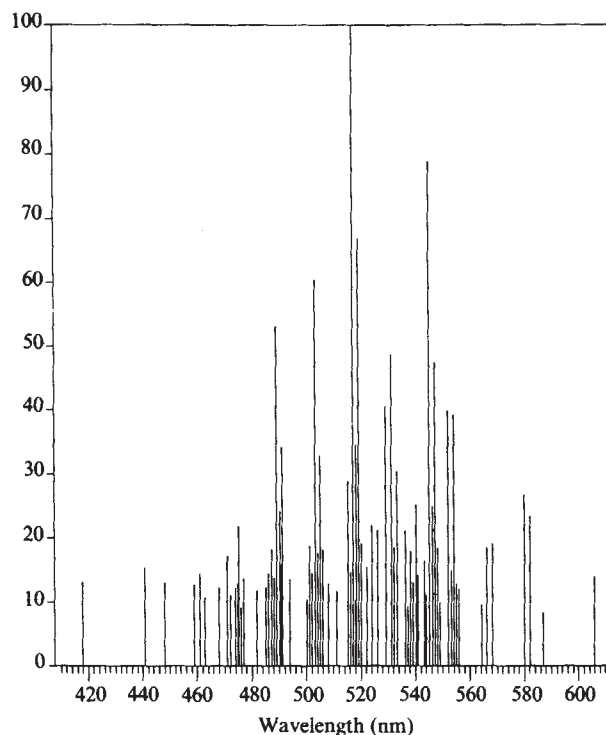
Table 1 Metal analysis of metalloporphyrin fraction isolated from Daw Mill coal

Metal	%
Gallium	5.7
Sodium	0.49
Iron	0.36
Potassium	<0.2
Magnesium	<0.2
Aluminium	0.17

Powdered dried coal (92 kg) from Daw Mill Colliery (South Midlands, Int. Code No. 811) was extracted with 7% v/v sulphuric acid in methanol for 12 h. The filtrate was diluted and much of the organic material was transferred into chloroform, which was washed (aqueous NaHCO_3 , water) and taken to dryness (42.8 g). This crude extract was submitted to column chromatography on silica gel, the elutant being benzene-methanol. Porphyrin-containing fractions were combined and submitted to preparative TLC on silica gel irrigated with benzene-ammoniacal methanol. Three red bands, barely separated, appeared at $R_F \sim 0.5-0.55$. These were combined, extracted and submitted to further purification by TLC. Finally the orange-red material was extracted with dichloromethane. The extract was filtered, concentrated and treated with petroleum ether to give the metalloporphyrin concentrate (17.8 mg, representing 0.19 p.p.m. of coal).

The metalloporphyrin concentrate thus obtained was a purple solid, not obviously crystalline but possessing a metallic sheen, and it did not melt below 300 °C. Metal analysis by thermal neutron activation (AERE, Harwell) showed that gallium was the principal metal present, with much smaller amounts of sodium, iron, potassium, magnesium and aluminium (Table 1). These latter metals possibly represent inorganic impurities, although iron and aluminium may both be present as metalloporphyrins.

A solution of the metalloporphyrin fraction in chloroform was red, and had a brilliant orange-red fluorescence in UV light (365 nm). The absorption spectrum (Fig. 1), with a Soret band at 405 nm, and visible peaks at 532 and 570 nm was typical of a metalloporphyrin, and resembled that of hydroxogallium(III) octaethylporphyrin ($\lambda_{\text{max}}^{\text{CHCl}_3}$ 404, 533 and 571 nm). The NMR spectrum accorded with that expected for a polyalkylporphyrin complex of a diamagnetic ion. The signals were broad, however, and this is attributed to the presence of a mixture of such complexes and to the effect of small amounts of paramagnetic ions.

**Fig. 1** Absorption spectrum of the metalloporphyrin fraction from Daw Mill coal. Solvent, chloroform.**Fig. 2** Mass spectrum of metalloporphyrin fraction from Daw Mill coal. The sample was introduced with a direct insertion probe, with a source temperature of 300 °C (measured at PCMU, Aldermaston).

The mass spectrum (Fig. 2) was very informative, and clearly indicated a mixture of homologous metalloporphyrins. The largest peak, m/e 517.185, corresponded to $\text{C}_{30}\text{H}_{32}\text{N}_4$ ^{69}Ga (the axial ligand having been lost), and was attended (natural distribution⁸ of gallium isotopes ^{69}Ga : ^{71}Ga = 60.4:39.6) by a second peak at m/e 519.188 ($\text{C}_{30}\text{H}_{32}\text{N}_4$ ^{71}Ga). These molecular formulae correspond to complexes of didemethyletioporphyrin, or an isomer of this. Some other components of this homologous series are given in Table 2, from which it is seen that the second most abundant pair of ions (m/e 547, 545) correspond formally to the gallium complex of tetraethyltetramethylporphyrin (etioporphyrin). Although each ion may well represent an isomeric mixture, the formulations suggested above are favoured on geobiogenetic grounds and the general analogy with the petroporphyrins¹. Support came from HPLC (Fig. 3) using a reverse phase system⁷ which proves to be very useful in the analysis and separation of polar porphyrins such as the gallium complexes. This revealed several components, one of which had the same retention time, within the resolution of the column, as synthetic hydroxogallium(III) etioporphyrin I.

Small-scale experiments on other coals gave similar but not identical results. Three other British bituminous coals (Annesley, South Nottinghamshire, Int. Code No. 633; Bersham, Western Region, Int. Code No. 634; and Linby, South Nottinghamshire, Int. Code No. 732) contained metalloporphyrins with similar properties, as did a Turkish lignite (Ankara-Beypazari). However, samples of a German brown coal and a Polish brown coal (Konin) contained a porphyrin fraction with different properties. With the amounts of porphyrin detected (<0.6 p.p.m.) it is important to appreciate the dangers of contamination, especially in a laboratory where porphyrins have been studied for many years. The different results obtained with the brown coal samples argue against this possibility, and it turns out that gallium porphyrins had not been prepared in our laboratory before this work. Moreover the gallium complex of octaethylporphyrin (a common ligand in our laboratory) was not present in the metalloporphyrin concentrate from coal (its

Table 2 Some accurately measured ions in the mass spectrum of the metalloporphyrin fraction from Daw Mill coal

Observed mass	Abundance	Formula	Calculated mass
547.220	47.3	C ₃₂ H ₃₆ N ₄ ⁷¹ Ga	547.219
545.218	78.7	C ₃₂ H ₃₆ N ₄ ⁶⁹ Ga	545.220
533.205	30.3	C ₃₁ H ₃₄ N ₄ ⁷¹ Ga	533.203
531.200	23.0	C ₃₁ H ₃₄ N ₄ ⁶⁹ Ga	531.204
519.188	66.7	C ₃₀ H ₃₂ N ₄ ⁷¹ Ga	519.188
517.185	100	C ₃₀ H ₃₂ N ₄ ⁶⁹ Ga	517.188
505.171	32.8	C ₂₉ H ₃₀ N ₄ ⁷¹ Ga	505.172
503.169	60.2	C ₂₉ H ₃₀ N ₄ ⁶⁹ Ga	503.173
491.157	34.0	C ₂₈ H ₂₈ N ₄ ⁷¹ Ga	491.156
489.155	53.0	C ₂₈ H ₂₈ N ₄ ⁶⁹ Ga	489.157
477.140	13.6	C ₂₇ H ₂₆ N ₄ ⁷¹ Ga	477.140
475.137	21.7	C ₂₇ H ₂₆ N ₄ ⁶⁹ Ga	475.141

Measured at PCMU, Aldermaston, on a Kratos (AEI) MS50 mass spectrometer with a DS-50SM data system. Direct insertion, 300 °C at 70 eV.

presence would have been revealed by a pair of peaks m/e 601 and 603 in Fig. 2).

Contamination with gallium is perhaps less likely. Nevertheless, we have felt it necessary to carry out control experiments in which the chromatographic materials were treated in appropriate conditions with trace quantities of octaethylporphyrin. The latter substance was recovered, but the gallium complex was not detected. Nor was gallium incorporated when basic gallium acetate was treated with octaethylporphyrin in 7% H₂SO₄-MeOH (18 h at room temperature, that is approximating to the conditions of the coal extraction). This experiment is important, as gallium is one of several trace metals present in coal^{9,10}; indeed, processes are described for the isolation of the metal from this source¹¹.

Because of the possibility of fractionation during the coal extraction, we do not consider that we have isolated all the metalloporphyrins present in the coal sample, and clearly several minor peaks in the mass spectrum remain to be assigned. These will be discussed elsewhere. The mass spectrum does suggest, however, that the ratio between gallium complexes of the etioporphyrin series ($M \pm 14n$, $M = 545$, 547) and the analogous complexes of the presumed 13²,15-cyclo-etioporphyrin III (deoxophylloerythroetioporphyrin) series ($M' \pm 14n$, $M' = 543$, 545) is generally high. This, and the low abundance of the metalloporphyrins in bituminous coal compared with that in many crude oil deposits, is consistent¹² with the more drastic thermal conditions believed to be necessary for coal to form. Indeed, it seems reasonable to suppose that coal rank may be inversely related to available metalloporphyrin content.

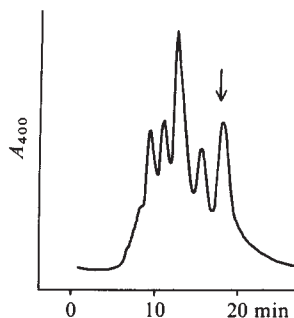


Fig. 3 HPLC of metalloporphyrin fraction on reverse phase column (Waters, Bondapak C₁₈/Porasil B) eluted with MeOH:H₂O = 72.5:27.5. The arrow indicates the component with a retention time indistinguishable from that of synthetic hydroxogallium(III) etioporphyrin. The column resolves homologues, not isomers, of polyalkylporphyrins.

The presence of gallium in coal porphyrins is unexpected: the abundance of gallium in the Earth's crust (15 p.p.m.) is an order of magnitude less than that of vanadium⁸, and, although some organisms accumulate gallium¹³, the biological function of this does not seem to be understood. The vanadyl porphyrins which occur in crude oil are thermodynamically and kinetically stable, and it may be argued that once formed they persist through geological time. Whether a similar argument can be applied to gallium porphyrins is not known: they are chemical rarities and require further study.

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No-flow equilibration and adsorption dynamics during ionic transport in soils

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Methods for predicting the movement of adsorbing chemicals such as fertilisers, pesticides, herbicides and heavy metals in soils have received increasing attention because of their importance in relation to the efficiency of fertiliser usage and in the control of agricultural and industrial pollutants¹. Because of the difficulty in coping with the complex non-linear partial differential equations necessary to describe the dispersive-convective-adsorptive flow process, analytical solutions have been largely limited to the assumptions of instantaneous equilibrium and simple linear adsorption isotherms²⁻⁵. We demonstrate here the need to incorporate adsorption dynamics to describe adequately practical situations, particularly those involving intermittent flow.

In reality the movement of water through soils rarely, if ever, occurs continuously or at uniform flow velocity for any length of time. For example, the water content of the soil described as 'field capacity'⁶ results from the much slower redistribution of water following infiltration. Consequently there is a period following infiltration when flow is negligible but adsorption/desorption processes may continue. Thus, rather than being able to assume a constant or average pore water velocity and instantaneous equilibration between solute and adsorbent, it is imperative to consider the effects of the 'go-stop' nature of water movement where adsorption dynamics are concerned.

The importance of this effect is illustrated by our recent studies of the influence of adsorption dynamics on the movement of adsorbing ions in soil columns. The term 'adsorption dynamics' is used here to include not only adsorption kinetics at

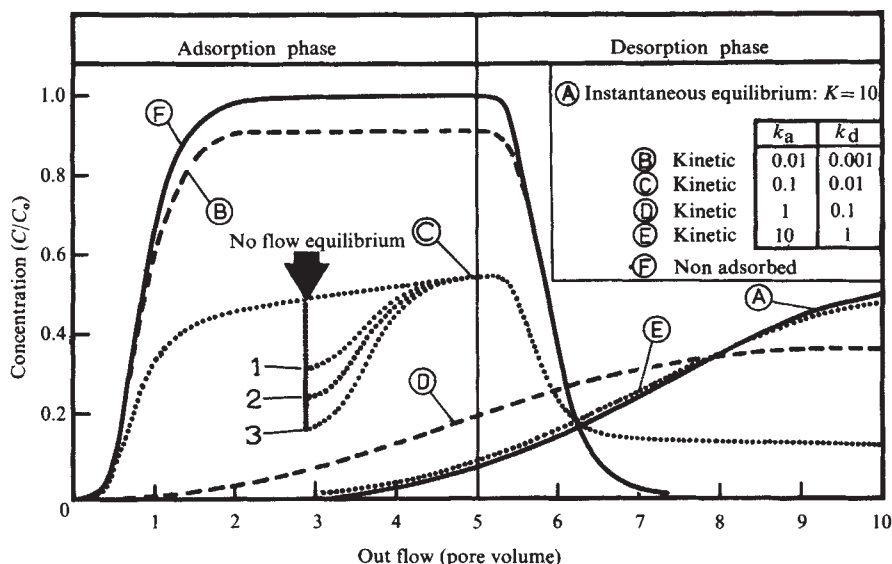


Fig. 1 Simulation of adsorption-desorption breakthrough curves for instantaneous equilibrium (curve A), kinetic models (B, C, D, E) and non-interacting solute (F). Effects of no-flow equilibrations are shown on curve C for increasing periods equivalent to the time taken for the outflow of 2, 4 and 10 pore volumes respectively.

solid surfaces, but also any intermediate steps which may influence the rate at which chemicals are exchanged between solution and adsorbed phases. In particular this includes diffusion from the centres to the walls of hydrodynamic pores (that is, pores responsible for hydrodynamic dispersion) but excludes intra-aggregate diffusion which can be viewed as a separate process.

A standard technique for studying the movement of ions in soil^{7,8} is the measurement of 'breakthrough curves' which describe the change in the outgoing solution from a soil column with outflow volume following a change in input concentration. Figure 1 contrasts the breakthrough curves, predicted by computer simulations using numerical methods, for non-instantaneous equilibration involving a range of different adsorption and desorption rates (k_a and k_d respectively), with that obtained for instantaneous equilibration (at the same equilibrium constant) using a simple linear isotherm. The simulations depict the passage of five pore volumes of solution containing the ion into a column of soil saturated with water (adsorption phase) followed by five pore volumes of ion free water (desorption phase). The value of the equilibrium constant K at relevant soil solution concentrations is generally greater than unity and $K = 10$ has been used as a suitable example.

The assumption of instantaneous equilibrium means that for two ionic species having the same or similar equilibrium isotherms, identically shaped and positioned breakthrough curves (curve A) will be predicted for the passage of their concentration pulses through a soil column. However, the rates of approach to adsorption and desorption equilibrium may vary considerably between different species and this will result in a range of markedly different breakthrough curves depending on the relevant rate coefficients (for example, curves B, C, D and E).

The consequence of different 'no-flow equilibration' periods on the breakthrough curves predicted in any case involving adsorption dynamics is particularly relevant. This is illustrated by the depressions in outgoing concentration for curve C corresponding to no-flow periods of increasing length. The assumption of instantaneous equilibration (curve A) will, of course, obscure this effect completely.

In the previous simulations simple linear adsorption kinetics were used to demonstrate the effects of time dependency of the adsorption/desorption processes. However, in other simulations we have shown that the same considerations are relevant regardless of the type of isotherm involved, for example, Freundlich and Langmuir.

The considerable practical significance of this effect is demonstrated by the breakthrough curves in Fig. 2 obtained for solutions of phosphate (2.5 mM), selenite (1.0 mM) and sulphate (1.0 mM) ions, and tritium labelled water including a

24 h no-flow equilibration period. The soils used were in a finely-ground state and the depressions in outgoing concentration for phosphate and selenite following no-flow periods cannot be attributed to intra-aggregate diffusion. This is verified by the absence of measurable depressions in the case of the sulphate despite the measurable adsorption of this ion and for the effectively non-adsorbed tritium ion. Thus it is clear that adsorption dynamics as previously defined, can be particularly important in controlling ionic transport in real conditions. The significant difference in the shapes of the breakthrough curves for sulphate and tritium, despite the absence of no-flow depressions for both, clearly demonstrates that adsorption kinetics form only part of the total adsorption dynamics influencing ionic movement. Other studies demonstrate that in aggregated systems the involvement of intra-aggregate diffusion before surface interactions, introduces no-flow depressions in outgoing concentrations for even non-adsorbed species and greatly enhances those for adsorbing species.

Such intermittent flow/no-flow measurements apparently provide a simple and convenient technique for checking the pertinence of adsorption dynamics for any particular ion-soil system, solution concentration and flow velocity, and we are not aware of any previous such demonstration in non-aggregated systems.

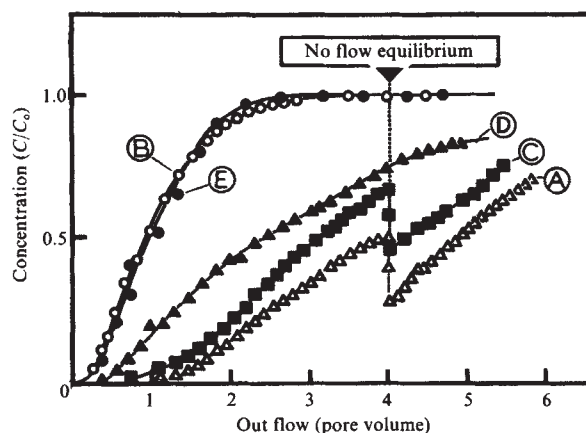


Fig. 2 Experimental breakthrough curves for phosphate (A) and tritium (B) movement through Yalanbee sandy loam and selenite (C), sulphate (D) and tritium (E) through York sandy loam showing effects of 24-h no-flow equilibration. Flow velocity equivalent to 0.3 pore volumes per hour.

The nature of the rate determining steps in these studies will require further investigation. While studies on equilibrium distributions involving agitation and high solution-to-solid ratios generally indicate extremely fast ion-soil interactions⁹, the present observations demonstrate that such measurements of adsorption kinetics may be of little relevance in relation to ionic transport. There is increasing evidence that considerably slower rates are involved in actual soil systems^{10,11}.

These observations form part of a much more detailed study of ionic transport through soil systems involving computer simulations using numerical methods and experimental determinations in soil columns being undertaken in this Department.

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3,360-Myr old gneisses from the South Indian Craton

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Reliably dated continental crust older than 3,000 Myr is only known from restricted segments of cratons in southern Africa, Greenland-Labrador, and Minnesota (summarised in ref. 1). These rare Archaean nuclei are very important in constraining the timing and mechanism of the differentiation of continental material from the mantle. We report here preliminary Rb-Sr whole rock data for Archaean gneisses from the Gorur-Hassan area, South Indian Craton which yield the oldest age yet reported from the Indian sub continent ($3,358 \pm 66$ Myr) with a very unradiogenic initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (0.7000 ± 0.0004) which implies that the igneous precursors of these gneisses were derived from a relatively Rb-depleted source within the mantle.

The gneisses of the Gorur-Hassan area (Fig. 1) are components of the polyphase Archaean Peninsular Gneisses of the South Indian Craton. They are unconformably overlain by the low grade volcanic-sedimentary association of the Dharwar supergroup, represented locally by the Bababudan and Shigegudda belts². Their relationship to the supposed pre-Dharwar, high grade volcanic-sedimentary association of the Holenarsipur belt^{2,3} is, as yet, ill defined. The areas to the west and east are characterised by marked late or post-Archaean shear belts in which gneisses and supracrustals are deformed together. To the north-west, the gneisses have undefined relations with a foliated granitoid mass, the Chickmagalur Granite, which is also unconformably overlain by the Dharwar supergroup of the Bababudan belt. Both the gneisses and the Holenarsipur supracrustals are intruded by a small post-kinematic granitoid mass the Mavinkere Granite, which may also post-date the major shear belts (Fig. 1).

The gneisses described here were chosen for reconnaissance dating because of their location in an area of low finite strain and the fact that they are stratigraphically the oldest known materials in the South Indian Craton readily amenable to the Rb-Sr

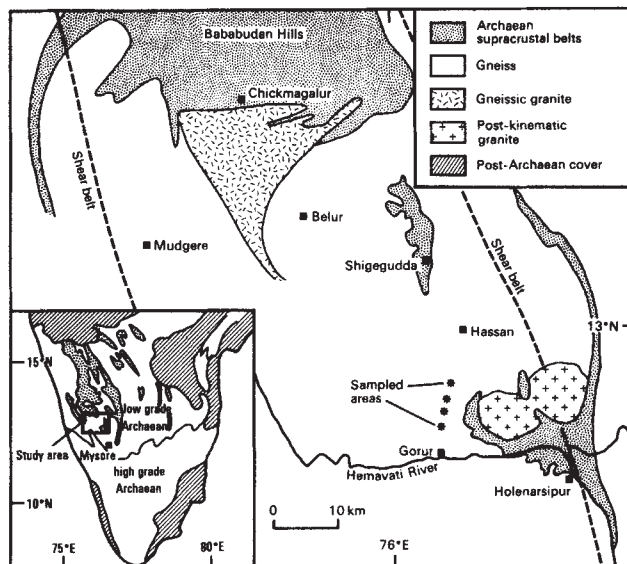


Fig. 1 Geological sketch map of parts of Hassan and Chickmagalur Districts, Karnataka.

method. The sample were all taken from active quarries and are extremely fresh rocks.

The gneisses range from dark dioritic amphibolites through tonalites and granodiorites to very pale trondjemites. This gives the gneisses a broad banded aspect, each band showing little evidence of strong deformation. In places the compositional banding is emphasised by parallel highly strained zones with isoclinal folds. Late stage epidotic crush zones have developed locally. Analysed samples were collected from the least deformed representative of each phase, and at least 3 km from the post-kinematic Mavinkere Granite (Fig. 1).

The Rb-Sr data set out in Table 1 were determined at IGS by standard methods of mass and X-ray fluorescence spectrometry using an automatic V.G. Micromass 30 and an automatic Philips PW 1450. Data for standards obtained during the course of the sample analyses are also set out in Table 1. The Rb-Sr data are plotted on an isochron diagram in Fig. 2.

Least squares regression of the data points using the method of Williamson⁴ yields a slope and intercept corresponding to an age and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of $3,369 \pm 31$ Myr and 0.6995 ± 0.0002 (1σ errors). The MSWD, indicating how well the points fit a line, using the 1σ errors estimated from replicate analyses (Table 1) is 15.2. Rejecting two samples, whose thin sections show slight epidotisation, reduces the MSWD to 1.84 and yields an age of $3,358 \pm 66$ Myr (2σ) and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7000 ± 0.0004 (2σ) from the five-point isochron. Work is in

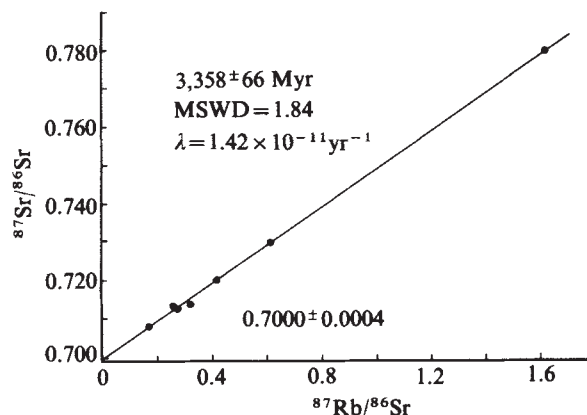


Fig. 2 Rb-Sr whole rock isochron plot for Gorur-Hassan gneisses. Errors are quoted at 2σ level.

Table 1 Rb-Sr analytical data for Gorur-Hassan gneisses

Sample no. and rock type	Rb (p.p.m. $\pm 5\%$)	Sr (p.p.m. $\pm 5\%$)	$^{87}\text{Sr}/^{86}\text{Sr}$ ($\pm 0.02\%$)*	$^{87}\text{Rb}/^{86}\text{Sr}$ ($\pm 1.0\%$)
I 291 grey-gneiss	56	966	0.70802	0.168
I 294 grey-gneiss	40	444	0.71300	0.259
I 285 biotite-trondjemite	39	270	0.72008	0.415
I 290 pale-gneiss	42	201	0.72966	0.611
I 284 biotite-granodiorite	36	64	0.77946	1.624
I 293 grey-gneiss	54	565	0.71257	0.275†
I 288 biotite-amphibolite	19	176	0.71359	0.318†
Data for standards Eimer and Amend			$^{87}\text{Sr}/^{86}\text{Sr}$	Rb/Sr (weight)
SrCO ₃ (19 runs)			0.70806 $\pm$ 1 (2 $\times$ s.e.)	
NBS 987 (14 runs)			0.71028 $\pm$ 2 (2 $\times$ s.e.)	
155725				0.691
GSP 1				1.100
G2				0.356
NBS 70A				8.053

* Normalised to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$ and corrected to be relative to value of 0.70800 for the Eimer and Amend SrCO₃ by subtracting 0.00006.

† Not included in regression shown on Fig. 2.

progress to document further the Rb-Sr systematics of this crustal segment.

Previous whole rock Rb-Sr isochron ages for the Peninsular Gneisses range from 2,640 (ref. 5) to 3,250 Myr (ref. 6). The latter age was derived from three gneissic clasts in the basal Dharwar conglomerates and consequently has gross errors and little geological significance. However, it does suggest that gneisses of the age we report here may be widespread in the South Indian Craton.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7000 ± 0.0004 lies slightly below an $^{87}\text{Sr}/^{86}\text{Sr}$ growth line for the mantle (Rb/Sr = 0.03) and well below most similar Archaean gneisses from elsewhere (Fig. 3). Assuming that the initial ratio of the gneisses grew from a primordial ratio of 0.699 (ref. 7) at 4,560 Myr to their measured value at 3,358 Myr the Rb/Sr ratio during this time must have averaged about 0.020. This implies a source region for the igneous precursors of the gneisses depleted in Rb compared with more typical mantle (Rb/Sr = 0.038). Such a depletion may reflect either: (1) an earlier differentiation of material with high Rb/Sr ratios from the mantle, in other words it is possible that even older crustal rocks may be identified in the South Indian Craton; (2) a mantle source region that was originally anomalously low in initial $^{87}\text{Sr}/^{86}\text{Sr}$ in comparison with basaltic achondrites or other meteorites (ref. 7); (3) a source region that

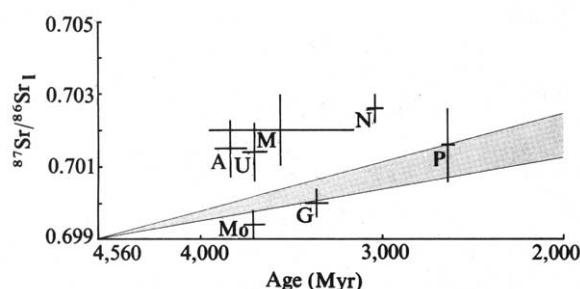


Fig. 3 Graph of initial $^{87}\text{Sr}/^{86}\text{Sr}$ versus age for Archaean gneisses relative to a range of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for igneous rocks derived from the mantle (stipple): A, Amitsog gneiss, West Greenland⁸; N, Nûk gneiss, West Greenland⁹; M, Mashaba gneisses, Rhodesia¹⁰; U, Uivak gneisses, Labrador¹¹; Mo, Morton gneiss, Minnesota¹²; P, Peninsular gneiss, Mysore⁵; G, Gorur-Hassan gneiss, this paper.

had been depleted in Rb/Sr by some indeterminate process. It is not possible to choose between these alternatives at present, although possibility (2) appears least probable.

The very low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio virtually rules out any evolution of the gneisses' Sr isotope system before the measured age. We therefore interpret the age of $3,358 \pm 66$ Myr to represent the age of separation of the gneisses' precursors from a mantle depth source region. The gneisses are the oldest rocks known from the Indian sub-continent. The anomalously low $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the gneisses and the reported presence of metapelitic and metabasic enclaves within the gneisses of the low finite strain zone of the Hassan District (B. Chadwick, personal communication) suggest the Hassan District to be potentially the most fruitful area for investigations of the early crustal history of the Indian sub-continent.

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Convergent metamorphism of eclogite and garnet-bearing ultramafic rocks at Lien, West Norway

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Eclogite and garnet peridotite and pyroxenite are important features in the supracrustal sequence of the Basal Gneiss region in West Norway. Three modes of origin have been proposed for these rock types: (1) both eclogite and garnet-bearing ultramafic rocks equilibrated in the upper mantle and subsequently were tectonically emplaced in their present positions¹⁻³, (2) the eclogites were produced by *in situ* metamorphism of a crustal sequence, but the ultramafic rocks originated in the upper mantle and were tectonically emplaced⁴⁻¹⁰, and (3) the ultramafic rocks were derived from the mantle but were emplaced before the eclogite facies metamorphic event and have shared a common metamorphic history, at least in part, with the crustal eclogites¹¹. I report here the application of recently developed geothermometers/barometers to garnet-bearing assemblages in the Lien peridotite at Almklovdaalen and to adjacent eclogites in surrounding supracrustal gneisses which has documented a retrograde metamorphic trend from 820 °C, 28.1 kbar to 645 °C, 17.6 kbar in the ultramafic rocks and a prograde trend in the eclogites which converges with that of the ultramafic rocks. The Lien peridotite seems to have been derived from the upper mantle in eclogite facies conditions and emplaced into unusually thick continental crust during an eclogite facies metamorphic event.

Table 1 Ranges in chemical composition of eclogite facies minerals in garnet peridotite, garnet pyroxenite, and eclogite

	Peridotite		Pyroxenite		Eclogite	
Olivine	87.8–91.9		88.4–91.0			
Fo						
Orthopyroxene	Cores	Rims	Cores	Rims	Cores	Rims
Wo	0.3–0.5	0.3–0.3	0.3–0.3	0.3–0.2		
Fs	10.8–7.7	10.9–8.1	12.7–8.0	12.6–9.4		
En	88.9–91.8	88.9–91.6	87.0–91.7	87.2–90.4		
Al ₂ O ₃ (wt %)	0.98–0.59	0.71–0.55	1.02–0.46	0.65–0.45		
Clinopyroxene						
NaCrSi ₂ O ₆	4.9–3.2	3.9–3.1	1.1–3.4	1.1–0.9	0.3–0.2	0.1–0.0
NaFe ³⁺ Si ₂ O ₆	1.3–1.8	1.5–1.8	2.1–1.2	1.8–1.6	4.5–2.3	4.4–4.6
NaAlSi ₂ O ₆	8.8–3.6	3.3–2.1	11.2–8.3	9.4–6.1	36.0–33.9	37.6–29.8
CaTiSi ₂ O ₆	0.1–0.2	0.1–0.1	0.2–0.1	0.2–0.0	0.2–0.3	0.4–0.4
CaAl ₂ SiO ₆	1.3–1.9	2.6–2.0	0.4–0.9	0.0–1.0	0.1–0.8	3.1–3.9
CaMgSi ₂ O ₆	76.3–81.3	80.7–83.7	76.0–80.3	78.6–84.0	52.1–53.8	47.5–54.7
CaFeSi ₂ O ₆	3.0–4.1	5.1–4.5	5.1–2.9	5.0–3.9	5.2–7.4	5.5–5.4
Mg ₂ Si ₂ O ₆	4.2–3.7	2.7–2.6	3.6–2.8	3.7–2.4	1.4–1.2	1.2–1.1
Fe ₂ Si ₂ O ₆	0.1–0.2	0.1–0.1	0.2–0.1	0.2–0.1	0.1–0.1	0.2–0.1
Garnet						
Py	61.9–69.3	59.9–69.2	59.2–71.9	58.6–70.1	21.1–35.1	29.0–40.8
Alm + Sp	22.5–18.1	27.0–18.3	29.2–16.1	29.7–17.9	55.0–45.0	43.1–39.3
Gr	12.6–12.6	13.1–12.5	11.6–12.0	11.7–12.0	23.8–19.9	27.9–19.9

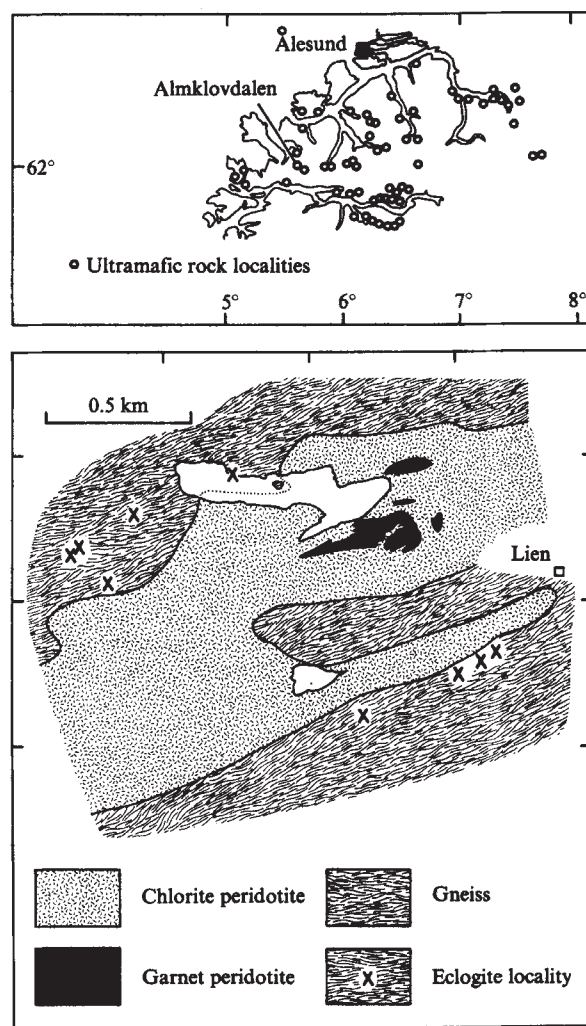
One of the classic occurrences of garnet peridotite and pyroxenite in west Norway is located within the Lien peridotite^{1–2,12–13} (Fig. 1), which is part of a large ultramafic body in Almklovdaalen¹⁴. The Lien peridotite consists predominantly of partly serpentinised, foliated chlorite peridotite containing an equilibrium assemblage of forsterite–enstatite–tremolite–clinocllore ± low Al chromite with a fine- to medium-grained mosaic texture. Four areas of garnet peridotite with locally abundant garnet pyroxenite layers are preserved within the Lien body (Fig. 1) and represent relicts of the earliest discernible mineral assemblage in the ultramafic mass. Garnet peridotite displays an inequigranular texture in which large strained grains of clinopyroxene, orthopyroxene, and garnet occur in a fine- to medium-grained mosaic matrix of strain-free olivine, orthopyroxene, clinopyroxene, pargasitic amphibole, and small amounts of chromiferous spinel. Garnet pyroxenite displays similar texture and mineralogy, except that olivine is subordinate in amount, or absent. In both rock types garnet is commonly surrounded by a kelyphitic rim of finely intergrown amphibole and spinel.

The Lien peridotite has generally concordant contacts with surrounding gneisses of tonalitic to granodioritic composition. Garnet- and muscovite-bearing layers are present in the gneiss, and amphibolite layers and boudins are common. Amphibolite facies mineral assemblages in the gneiss are compatible with the chlorite peridotite assemblage in the ultramafic rocks, which is stable from 700 °C to 780 °C (ref. 15).

The predominant structural style in gneiss and chlorite peridotite is one of tight to isoclinal similar folds with a penetrative axial plane foliation which strikes east-northeast and dips steeply to the south. Linear elements of these folds measured in both rock types over the map area plot along a girdle which coincides with orientation of the axial plane foliation in the area. Thus, not only do gneiss and chlorite peridotite contain compatible mineral assemblages, they also share the same structural elements.

Nine occurrences of eclogite boudins, the largest of which is 20 m by 50 m, have been located in gneiss adjacent to the Lien peridotite (Fig. 1). Eclogite contains a primary mineral assemblage of garnet–omphacite–edenitic amphibole ± zoisite ± biotite–quartz–rutile–apatite–sulphide ± carbonate. Retrograde recrystallisation has been extensive in some samples, largely due to development of amphibole–plagioclase symplectite from omphacite and amphibole kelyphite from garnet.

Electron microprobe analyses were obtained for cores and rims of minerals in five samples of garnet peridotite, seven of

**Fig. 1** Locality and geologic map of the Lien peridotite in Almklovdaalen, west Norway.

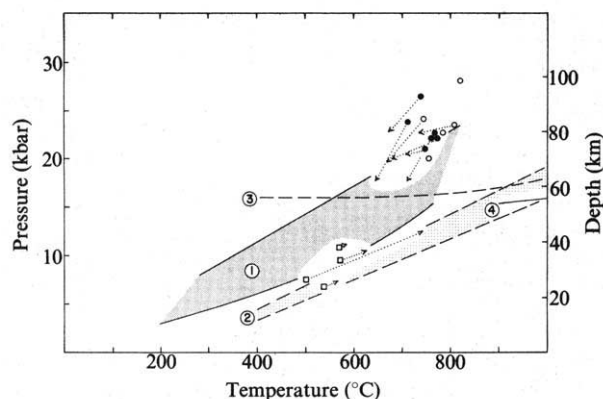


Fig. 2 Estimates of pressures and temperatures for garnet peridotite, garnet pyroxenite, and eclogite at Lien. Stippled area 1, prograde metamorphic trend for eclogites in Basal Gneiss of west Norway⁸; stippled area 2, field for transition of basic granulite to eclogite⁴; 3, boundary separating stability fields of garnet lherzolite and spinel lherzolite²⁰; 4, garnet lherzolite-spinel lherzolite transition in the system CaO-MgO-Al₂O₃-SiO₂ (ref. 21). ○, Core compositions of minerals in garnet peridotite; ●, cores in garnet pyroxenite; □, cores in eclogite; arrows, rim compositions of minerals.

garnet pyroxenite, and four of eclogite (summarised in Table 1). Fe³⁺ was determined by charge balance calculations and by wet chemical analyses of selected mineral separates.

Coarse-grained garnet, clinopyroxene, and orthopyroxene in garnet peridotite and pyroxenite are compositionally zoned; from core to rim there is an increase in Fe and decrease in Mg in garnet, a slight decrease in Al and Cr in orthopyroxene, and a decrease in Al, Cr, and Na in clinopyroxene. Compositional zoning in eclogite garnet is opposite to that in peridotite and pyroxenite, that is, a decrease in Fe and increase in Mg from core to rim. Zoning in eclogite clinopyroxene is slight, there being a small increase in Al and decrease in Ca from core to rim.

Due to compositional zoning $K_{D}^{Fe/Mg}$ increases from cores to rims in peridotite and pyroxenite, reflecting a decrease in temperature during mineral growth, whereas $K_{D}^{Fe/Mg}$ decreases from cores to rims in eclogite, indicating an increase in temperature.

Estimates of equilibration temperatures for core and rim compositions of coarse-grained minerals in garnet, peridotite and pyroxenite have been obtained from geothermometers based on Fe-Mg partitioning among garnet, orthopyroxene, clinopyroxene, and olivine^{16,17} and on the pyroxene miscibility gap¹⁸. In Fig. 2 temperatures based on $K_{D}^{Fe/Mg}$ have been selected because this is the most sensitive of the various geothermometers in the temperature range of interest. Pressures of equilibration have been calculated according to Wood's¹⁹ procedure, based on the Al content of orthopyroxene in equilibrium with garnet and incorporating all corrections for Cr and Fe³⁺. Calculated values of pressure and temperature are consistently lower for rim compositions compared to core compositions in garnet peridotite and pyroxenite and reveal a clear trend of retrograde metamorphism from 820 °C, 28.1 kbar to 645 °C, 17.6 kbar, which lies within the stability field of garnet peridotite^{20,21}, but which penetrates the higher *P-T* portion of the prograde metamorphic trend for eclogites in this region established by Krogh⁸ (Fig. 2). Although pressure estimates may be in error up to ± 5 kbar¹⁸ due to uncertainties in temperature estimates, extrapolation of high temperature experimental results to lower temperature conditions, and the effects of relatively high concentrations of Cr in garnet and pyroxenes, it seems most likely that the Lien garnet peridotite and pyroxenite equilibrated in response to decreasing pressures of the order of 25–20 kbar and certainly <30 kbar. The *P-T* estimates obtained in this study are in close agreement with those reported recently by Carswell and Gibb¹¹ for Norwegian crustal garnet lherzolites.

Neither orthopyroxene, phengite, nor primary plagioclase occur in eclogites close to the Lien peridotite, and consequently, only minimum estimates of equilibration temperatures and pressures can be made²². Core and rim compositions of adjacent garnet and omphacite grains in four eclogite samples define a prograde metamorphic trend (Fig. 2), based on experimental calibration of Fe-Mg partitioning between garnet and clinopyroxene in basic rocks^{22,23} and the jadeite content of omphacite²⁴. Considering that only minimum pressure estimates can be made for these samples, their metamorphic pattern is consistent with that for other eclogites in the region. Furthermore, values for $K_{D}^{Fe/Mg}$ in the four eclogite samples (5.2, 8.0, 9.9, and 10.7) are compatible with values (5.5–9.5) which would be expected in basic rocks recrystallised in the same *P-T* conditions as those for the Lien garnet peridotite and pyroxenite.

This investigation supports a dual origin for eclogite and garnet-bearing ultramafic rocks at Lien, eclogite having developed by *in situ* metamorphism of crustal materials and ultramafic rocks having been derived from the upper mantle. Such an interpretation was proposed previously by Bryhni *et al.*¹⁰ and Carswell⁹ and subsequently supported by Brueckner's²⁵ strontium isotope analyses of clinopyroxene from eclogite and garnet-bearing ultramafic rocks. The metamorphic trends for eclogite and ultramafic rocks suggest that eclogite facies metamorphism of the ultramafic rocks may have begun in the upper mantle and then continued at progressively decreasing temperatures and pressures as the ultramafic rocks were emplaced in continental crust at depths of 60–70 km and that eclogite and garnet peridotite and pyroxenite have shared in the same metamorphic episode.

This investigation and published isotopic data indicate the following three-stage evolution of the Lien peridotite: (1) emplacement of mantle-derived garnet-bearing ultramafic rocks into a deep crustal environment and convergent metamorphism with eclogite 1,850 Myr ago²⁶; (2) extensive recrystallisation of the Lien peridotite and surrounding gneisses in amphibolite facies conditions 1,150 Myr ago²⁷; and (3) isotopic re-equilibration of the gneisses and development of hydrous fracture and contact mineral assemblages in peridotite during the Caledonian Orogeny^{27,28}.

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Size constancy fails below half a degree

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Size constancy is a term used to refer to the fact that objects seem to maintain size as their distance from an observer changes, despite change in image size. It is known that size constancy has limits^{1,2}. People seen from the top of tall buildings look small. By observing objects, including the Moon, and after-images, we demonstrate here that size constancy holds when image size is above half a degree, and breaks down when it is less. In other words, image size is scaled for distance only when image size is above half a degree.

The primary evidence for size constancy is negative: objects do not seem to change in size with change in their image size. An immediate experimental difficulty is that observers can judge both object size and image size separately. An observer who is asked if an object seems to change size as it moves away from him may reply 'yes', meaning, not that the object changes in apparent size, but that it covers a smaller patch of the visual field. For this reason, measurements obtained from naive subjects are particularly suspect in the field of size constancy. It is safer, therefore, to rely on observations that the reader can make and repeat himself, rather than what was said by experimental subjects, who may well have been confused about what was asked of them.

It may readily be observed, without the aid of instruments, that large objects begin to look small when their image size reduces to half a degree, or less. A convenient reference is the fingernail of the index finger, which subtends about a degree with the arm fully extended. People seen in the distance seem doll-like² when their image size is less than half the size of the image of the fingernail. Aeroplanes, after taking off, maintain object size and then, at a certain point, look small. The change occurs when image size shrinks to half a degree. Similarly, motor cars, lorries, trains and houses, seen from the air, look small, like toys, when their images subtend less than half a degree. As a plane comes in to land a passenger may observe the sudden transition from small to large occurring for each object as its image size breaks the half a degree barrier. Many other examples will be noticed by the reader as soon as he is alerted to half a degree as the critical image size, and learns to measure image size with his fingernail. The situation is less clear with smaller objects, although to the authors, household and laboratory objects look to have diminished in size at a distance at which each first subtends less than half a degree.

The proposition that size scaling holds only when image size is above half a degree may be tested by changing apparent distance while holding image size constant. One convenient method of so doing is to form an after-image, and observe it as projected on to surfaces at different distances. Emmert's law³ states that the apparent size of an after-image varies directly with the perceived distance of the surface on which it is projected. We have observed that Emmert's law breaks down when after-image size is less than half a degree. An after-image does not seem to change size with the distance of the surface on which it is projected when it is below the critical size of half a degree. The reader is urged to verify this observation, which he can do conveniently by observing the after-image formed by a flashgun, from a distance at which it subtends half a degree or less.

An alternative way of demonstrating this to a large group of people, which we have used several times, is as follows. A flashgun is so positioned that it subtends more than half a degree to roughly half the group, and less to the other half. All are asked to observe the after-image on the palm of the hand and then on a side wall of the room. They are then asked to raise their hands if the image seemed to change in size when transferred from the

near to the far surface. With high probability, subjects with images above half a degree (those in the front of the room) raise their hands and subjects with images below half a degree (those in the back of the room) do not, despite the fact that they can see raised hands in front of them. Results are well beyond chance levels, so much so that the experiment may be used as a classroom demonstration of Emmert's law and of its boundary condition.

The Moon is of interest in this connection, because it subtends almost exactly half a degree, whatever its position in the sky, and because it seems large at the horizon and small when high in the sky. Variation in apparent distance is the most probable cause⁴ of the Moon's variation in apparent size. According to the hypothesis that size scaling occurs at half a degree and above, but not below, the illusion of change in the Moon's size should be abolished by reducing the size of image. This can be done optically. The full Moon was observed in a clear sky at Easter, 1978 (and subsequently) through a positive lens close to the eye, and a negative lens further away (reversed galilean telescope), both contained in a tube and so positioned that the Moon's image was reduced by about one-third, to 20 arc min. The horizon Moon seemed to diminish by much more than a third in diameter, by a factor of two or three. The zenith Moon, when it was observed, seemed to diminish by only the expected one-third, like terrestrial objects whose distance can be judged by eye. Furthermore, the zenith Moon, when reduced, seemed to be of the same size as the horizon Moon, when it was reduced. These effects are not due to observing through a tube. The horizon Moon seemed much larger than the zenith Moon when both were observed through a tube containing no lenses. It may be relevant that, of all Jupiter's moons, only one, Io, subtends a visual angle near half a degree at Jupiter's surface. Thus, only Io should seem to change size to an observer on Jupiter.



Fig. 1 Because the figure in the background seems more distant than the small figure in the left foreground, its size seems larger when the page is at normal reading distance. At this distance both figures subtend more than 30 arc min in the vertical. At a greater distance, when both subtend less than 30 arc min, the two seem to be the same size. The head of the large figure also looks smaller than the Moon, which can be seen on the right of the picture. The head and Moon also seem the same size when they subtend 30 arc min or less. As the observer moves back from the picture, this critical image size is reached earlier for the Moon and the head than it is for the two smaller figures.

A final example of the fact that image size is critical is provided by Fig. 1. Three men are depicted (after Van Gogh), two drawn to the same size. Perspective makes the one in the background seem further away, and larger at normal reading distance. However, when the page is removed to a distance (about 3 m) at which both figures subtend 20 arc min, they seem the same size.

There may be some significance in the fact that size constancy breaks down at half a degree. It has been proposed^{5,6} that there are no size tuned channels in the low frequency range, but instead a single mechanism sensitive to luminance gradients: above the peak of the contrast sensitivity function there is harmonic analysis and below it contrast gradient analysis. The hypothesis may therefore be advanced that large and small retinal images are calibrated in different ways, the large being subject to automatic size correction to take account of distance, and the small giving absolute size information, which is not corrected for distance.

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A dorso-ventral shift of embryonic primordia in a new maternal-effect mutant of *Drosophila*

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Mutations altering the spatial pattern of embryonic primordia are valuable tools for studying the earliest steps of embryonic pattern formation. As the spatial organisation of the egg cell arises during oogenesis, such mutants are likely to affect the embryo via the maternal genome (maternal effect). In *Drosophila*, most maternal-effect mutants studied so far lead to early developmental arrest. A few others yield pattern defects apparently linked to local failure of blastodermal cellularisation rather than to abnormal arrangement of primordia or ooplasmic determinants^{1,2}. A small number of mutants, however, are known in which the embryonic fate map is affected, for example, *bicaudal*^{3,4}, which profoundly alters the antero-posterior pattern of embryonic organisation. The *bicaudal* phenotypes suggest that the mutation causes a perturbation of a morphogen gradient that defines the antero-posterior coordinate in early embryonic pattern formation^{5,6}. A new maternal-effect mutant, *dorsal*, seems to exert a similar influence on the dorso-ventral coordinate for patterning⁶. *Dorsal* (*dl*) has both a recessive and a dominant maternal-effect phenotype. The *dl* recessive phenotype is marked by an apparently complete lack of dorso-ventral polarity from early embryogenesis onward⁶. The *dl* dominant (*dl*^D) phenotype, which we report on here, is temperature sensitive: at 22 °C, *dl*/+ females lay eggs which develop normally while eggs produced at high temperature (29 °C) yield abnormal larvae which fail to hatch. These mutant *dl*^D larvae vary in phenotype but nearly always lack muscles and frequently part of the ventral hypoderm. They are defective in structures that arise from the ventral region of the blastoderm in normal embryogenesis^{6,7} (Fig. 1).

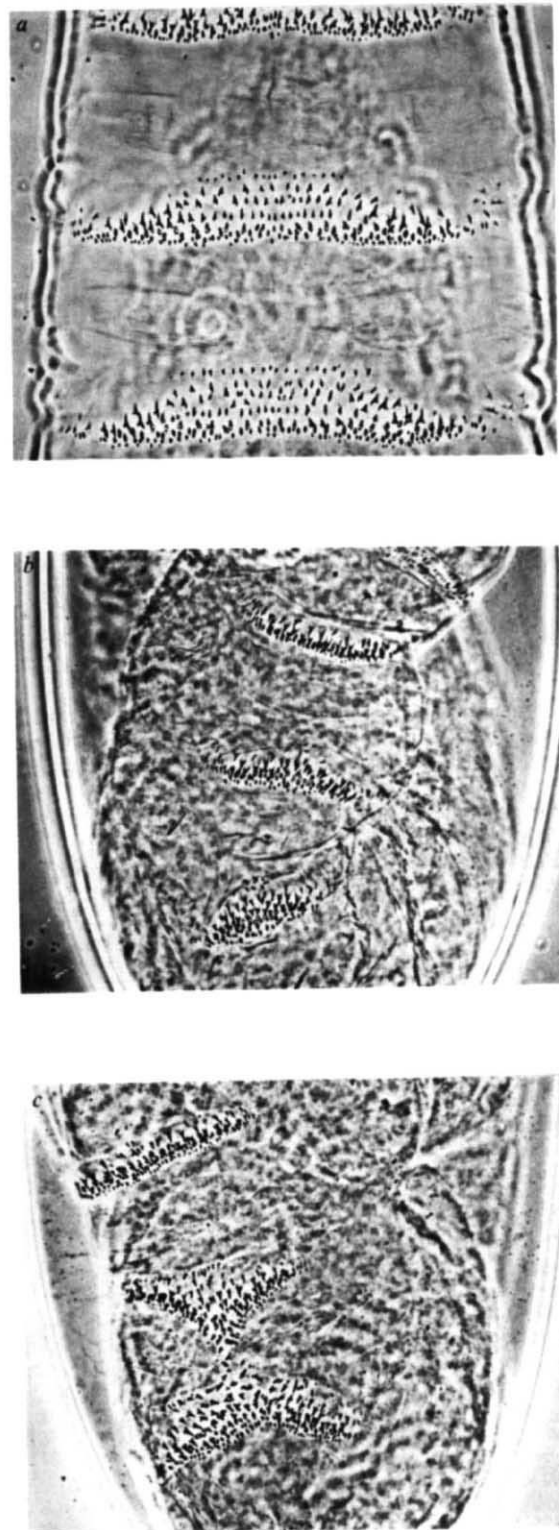


Fig. 1 Ventral hypoderm from the abdomen of wild-type and mutant larvae. a, Larva shortly after hatching; b, c, *dl*^D larvae produced by *dl*/+ females at 29 °C. The genotype of the females was *al dp b dl bic sp/vg*^D in all experiments. For the descriptions of genes see refs 6, 10. The expressivity of the *dl*^D-phenotype is dependent on the genetic background of the female and this particular genotype was chosen because it happened to produce weak and extreme phenotypes with approximately equal frequency, while less than 1% of the eggs hatch. Larvae were regarded as extreme mutant if they had a notably reduced width of the ventral denticle hook rows (b); they also always were twisted or coiled up in the egg case. The larva in c has been irradiated mid-ventrally at 40% egg length (0% = posterior pole) at the cellular blastoderm stage.

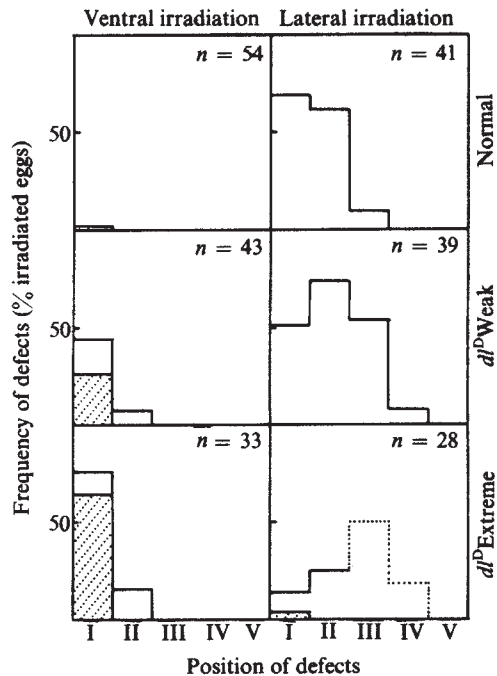


Fig. 2 Distribution of defects in the ventro-dorsal hypoderm pattern following UV-laser irradiation of wild-type and mutant eggs at the blastoderm stage. Eggs were collected from *dl/+* females (*al dp b dl bic sp/vg*^D) or *+/+* (Oregon K) females at 29 °C or 25 °C respectively in 30-min intervals, aged until briefly before blastoderm formation, partly dechorionated and irradiated with a UV-laser microbeam (257 nm, 20 µm diameters, 7 erg) at the cellular blastoderm stage as described⁹. Ventral irradiations (left panels) were aimed at the ventral midline of eggs orientated with their ventral side facing the UV beam. For lateral irradiations, laterally orientated eggs were irradiated at 30% egg diameter measured from the ventral side (vd4 in ref. 9). These positions correspond to 0% or 36% half-circumference (0% = ventral midline, 100% = dorsal midline). All eggs were irradiated at 30% egg length (0% = posterior pole). This position was chosen because it creates defects predominantly in the posterior abdomen (centre of defect 5th abdominal segment), a region permitting optimal detection of defects also in extreme mutant larvae which often are twisted or coiled in the anterior abdominal and thoracic region. Irradiation at more anterior position (40% and 50% egg length) gave similar results (data not shown). Following irradiation, more than 90% of the eggs survived to the larval stage. The larvae and unhatched eggs were fixed and cleared and the cuticle examined for defects using phase contrast optics at ×400 magnification (Zeiss Neofluar). For quantitative evaluation of the location of the defects the ventro-dorsal cuticular pattern was divided into five sections⁹ (I and II, ventral and lateral half of the ventral hypoderm; III, lateral hypoderm; IV and V, lateral and dorsal half of dorsal hypoderm, see ref. 9 for description of markers used). The frequency of defects per section was determined whereby defects spanning more than one section were counted separately for each section. Mutant larvae were regarded as extreme if the width of the ventral denticle belts was markedly reduced. Owing to a severe twisting of the hypoderm in extreme mutant larvae, the lateral and dorsal hypodermal region (sections III to IV) could sometimes not reliably be scored, the values indicated by stippled bars are therefore lower limits of the true defect frequency. The shaded bars indicate the frequency of bilateral defects, in which both right and left sides of the ventral hypoderm were affected simultaneously. No correction was made for spontaneous defects (<5% in both mutant and wild-type larvae), however, larvae with defects outside the abdominal segments A3–A7 (<5%) were disregarded in the calculations.

The absence of ventrally derived structures in *dl*^D embryos is not due to a failure of cell formation in ventral egg regions. The embryos develop an apparently normal cellular blastoderm. At gastrulation, the first deviation from normal development is observed: *dl*^D embryos fail to form the ventral invaginations which lead to the separation of the mesodermal and anterior

endodermal anlagen from the ectoderm in normal development⁶. The defect of ventral structures could thus result from an inability of the ventral cells to develop beyond blastoderm. Alternatively, the ventral cells could give rise to structures normally derived from more lateral egg regions, which would mean that the dorso-ventral fate map is affected by the mutation. To test this idea, we investigated the developmental fate of blastoderm cells in *dl*^D embryos. As a method for fate mapping, we used UV-laser induced local lesions.

We have previously shown that irradiating wild-type blastoderms with a UV-laser microbeam⁸ frequently leads to localised cuticle defects in the ensuing larvae⁹. The position of the defects within the cuticle pattern depends rather strictly on the site of irradiation, indicating that the defects were created by damaging the respective anlage cells. We have used this correlation to construct a detailed fate map of the larval epidermis⁹ (Fig. 3). The mid-ventral region (20% of the egg circumference) gives rise to mesoderm⁷ and irradiation of this region very rarely induces cuticular defects. The larval hypoderm stems from the larger part of the blastoderm (76%); ventro-lateral and lateral irradiations create defects predominantly in the ventral hypoderm, while the dorsal hypoderm is frequently defective following dorso-lateral and dorsal irradiations.

When *dl*^D embryos are irradiated as blastoderms, the pattern of the ensuing defects is markedly changed (Fig. 2). Following mid-ventral irradiations, 79% (26/33) of the extreme mutant larvae show a defect in their ventral hypoderm while only 2% (1/54) of the control larvae do so. Larvae of a weak mutant phenotype give an intermediate value (20/43). Moreover, the majority of defects are bilateral defects, impairing right and left side of the ventral hypoderm simultaneously, while ventral hypodermal defects created in normal larvae are always restricted to one side, consistent with the separation of the right and left ventral anlagen by the mesodermal anlage. This result

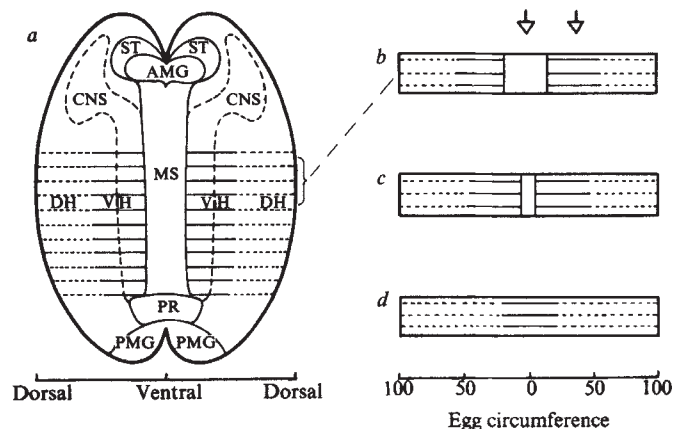


Fig. 3 Alteration of the embryonic fate map in *dl*-dominant embryos. *a*, Poulson's fate map⁷ of the *Drosophila* egg cut open along the dorsal midline and spread out. Simplified and slightly modified^{9,11}. The location of the segment borders in the larval hypoderm as determined from laser-induced lesions⁹ are indicated by the transverse lines, dotted for the dorsal part of the segments. The serosal anlage is not indicated separately because it seems that at least part of it is later incorporated in the dorsal hypoderm. AMG, anterior midgut; PMG, posterior midgut; ST, stomodeum; CNS, central nervous system; PR, proctodeum; MS, mesoderm; VH, ventral hypoderm; DH, dorsal hypoderm. *b–d*, Proposed alteration of the fate map in *dl*-dominant embryos; *b*, middle region of fate map in a normal embryo; *c*, in a *dl*^D embryo with weak; and *d*, with extreme mutant phenotype. The left and right anlage for the ventral hypoderm, flanking the mid-ventral mesodermal anlage, are shifted ventrally at the expense of the mesoderm anlage. The absence of anterior midgut and malpighian tubules (part of the posterior midgut⁷), in extreme mutant embryos suggests that the shift of lateral primordia towards the ventral midline also occurs at the anterior and posterior egg regions. The arrows indicate the sites of irradiation with respect to the egg circumference.

suggests that ventral cells which form mesoderm in normal development, instead form ventral hypoderm in *dl^D* embryos.

Lateral irradiations indicate that not only the fate of ventral, but also that of lateral cells is affected in mutant embryos. Figure 2 shows that in contrast to the high defect frequency in control embryos (88%) relatively few *dl^D* embryos with extreme phenotype have defects in their ventral hypoderm (7/28), most of the defects being located in the dorso-lateral part of the hypoderm. This shift of defect location towards more dorsal positions in the hypodermal pattern is less pronounced in weak mutant phenotypes. Note that in both ventral and lateral irradiations, the antero-posterior positions of the defects are very similar in mutant and wild-type embryos and correspond to the site of irradiation.

The results suggest that the absence of ventral structures in *dl^D* embryos results from a change of the embryonic fate map: ventral cells form ventral hypoderm instead of mesoderm, while lateral cells form hypodermal structures that are normally derived from more dorsal positions. It seems that the lateral and dorsal anlagen are shifted towards the ventral midline at the expense of ventral anlagen. The degree of this shift parallels expressivity of the mutant phenotype. In extreme cases, it may be as much as 30% of the dorso-ventral coordinate as judged from the width of the remaining ventral hypoderm (Fig. 1) and

by comparing the distribution of defects following lateral irradiations of extreme mutant larvae to that obtained in normal larvae by more dorsal irradiations⁹. The proposed alteration of the fate map is shown in Fig. 3.

We suggest that *dorsal*, rather than eliminating a localised determinant required for proper ventral development, affects a continuous property, such as a morphogenetic gradient, that defines the entire dorso-ventral coordinate of the developing embryo.

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Intrinsic immunosuppressive activity of different trypanosome strains varies with parasite virulence

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The virulence of African trypanosomes varies greatly with the host and parasite strain combinations used¹. In mice, infections range from those which are rapidly fatal to those which more closely resemble the chronic disease patterns seen in man and domestic cattle². The basis of trypanosome virulence is assumed to be related to intrinsic parasite replication rate and the effectiveness of the host's immune response to successive waves of parasites which differ in their surface antigens. Continued control of each emergent population requires sustained immune vigilance. Thus, the generalised immunodepression which accompanies African trypanosomiasis in man³, cattle⁴ and experimental animals^{5,6} would be expected to determine the fatal outcome of most untreated infections if the secondary immunodeficiency extends to responses against the parasite as well as to unrelated antigens. Virulence, in this view, could be explained by the rapidity and intensity with which a given strain of trypanosomes induces immunodepression in a given host. We have attempted to define the relationship between parasite virulence and generalised immunodepression by examining the intrinsic potential of trypanosome strains of differing virulence to induce immunodepression in the same murine host. Following the observation that killed trypanosomes or their subcellular membrane fractions mimic the immunological consequences of active infection *in vivo*⁷, it has now become possible to compare the immunosuppressive potential of acute and chronic strains by using active fractions derived from identical numbers of killed organisms. We conclude from these comparisons that trypanosome strains can have inherently different immunosuppressive activities, and that the extent to which IgM responses, in particular, are suppressed correlates with and suggests a basis for parasite virulence.

The parasitaemia profiles of four clones from three strains of *Trypanosoma brucei brucei* (see Fig. 1 legend) ranging in virulence from acute (NIM 9) to subacute (NIM 2 and 6) and chronic (NIM 7), are presented in Fig. 1. The association of fluctuating parasitaemias with the emergence of antigenically distinct parasites has already been described for clones NIM 2 and 6 (ref. 2) and for the chronic strain from which clone NIM 7 is derived⁸. Taken as a whole, the greater the level of initial parasitaemia, the shorter the survival time of the infected mice. In addition, the prepatent period, that is, the time from inoculation until first detectable blood parasitaemia, was shortest for clone NIM 9, for which even the initial parasitaemia was uncontrolled. The subacute and chronic clones each had indistinguishable prepatent periods of between 3 and 4 d, suggesting that their intrinsic replication rates were similar. This was confirmed by infection of irradiated mice in which no significant difference was observed between growth rates of NIM 2, 6 or 7 parasites in either blood or tissues (data not shown). Growth of clone NIM 9 was, as expected, significantly more rapid than that of the other clones in irradiated animals.

Membrane fractions derived from carefully determined numbers of organisms of each clone were prepared and injected into mice 4 d before priming with sheep red blood cells (SRBC). A large proportion of trypanosome material is routinely lost during the fractionation procedures. However, the final membrane yield was comparable between each of the four clones; reproducibly, 20–30 µg protein was derived from 10⁷ trypanosomes. The comparative suppressive effect of each fraction on the direct and indirect anti-SRBC plaque-forming cell (PFC) response is shown in Fig. 2. IgG response, measured by indirect PFC, seemed to be extremely sensitive to the effects of all four clones. Membrane fractions derived from 10⁸ parasites of each of the four clones inhibited up to 98% of the normal IgG response. Fractions from 10⁷ parasites were slightly less immunosuppressive, but the degree of suppression was still the same for all four clones. However, at this same dose, we were able to detect a strict hierarchy with respect to inhibition of IgM responses measured by direct PFC. The pattern of IgM suppression induced by the membrane fractions was directly correlated with the virulence of the trypanosome strains from which they had been derived. At a log higher dose, the acute and subacute clones each had potent suppressive effects (70–80%), whereas the inhibitory activity of the chronic strain remained significantly lower (35%).

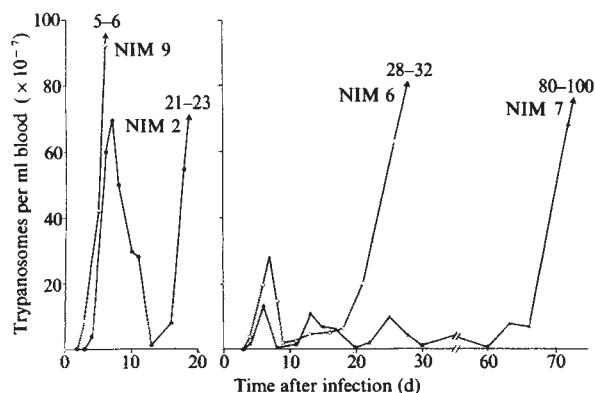


Fig. 1 Course of infection with four clones of *T.b. brucei* in (CBA/H×C57BL/6)_F₁ female mice, and survival times. Each point represents the mean parasitaemia of five or six mice, except for NIM 7, which represents the parasitaemia of a representative individual mouse. Trypanosome passage and cloning, and infection of mice with 200 parasites, were as described previously¹². Clones NIM 2 and NIM 6 are non-cross-reacting variant clones, both derived from primary isolate Serengeti/66/SVRP/42 (ref. 2), previously referred to as strain S42. Clone NIM 7 is derived from primary isolate Uganda/66/EVE/10, and NIM 9 is derived from primary isolate Uganda/60/EATRO/217. The nomenclature follows that recently proposed for the reference of salivarian trypanosomes¹³. Parasitaemias were followed by examination of wet tail blood films under high power and counts converted to trypanosomes per ml as previously described¹².

The comparative suppression induced during active infection is summarised in Table 1. When mice were infected with 200 parasites of clones NIM 2, NIM 6 or NIM 7 and challenged with SRBC 10 d later, the IgG antibody response was completely inhibited regardless of the relative virulence of the infecting clone. The suppression of IgM antibody was virtually complete (background levels) for those clones which led to uncontrolled parasitaemia soon after day 10. Only the IgM response of the chronically infected mice, which continued to control parasitaemia for up to 80 d, survived at least partially intact (17% of normal response).

Although these data are concerned with the effects of trypanosomes on the response to an unrelated antigen, a similar pattern of suppression of the response to the parasite itself is suggested by the findings that the intrinsic differences between *T. brucei* clones in suppressing primary immune responses, particularly IgM responses, correlate well with trypanosome virulence, and the extent of IgM suppression induced during infection correlates with the control of parasitaemia and the pattern of infection in that host.

Whereas IgG responses are readily and comparably eliminated by active fractions of all four clones regardless of virulence, IgM responses are less susceptible to inhibition, and the degree of suppression varies directly with parasite virulence. In infected mice, our results and those of Hudson and Terry⁸ essentially reflect these suppression patterns; thus, heterologous IgG responses are rapidly eliminated during both acute and chronic infections, whereas residual IgM responses persist during chronic infections in association with continued control of parasitaemia. These findings suggest that control of circulating trypanosomes can be achieved in the absence of IgG antibody by low levels of IgM. There is direct evidence to support this view. The control of blood parasites by nude mice occurs without detectable levels of IgG antibody⁹, and very low concentrations of IgM antibody have been shown to protect mice passively against infection with *Trypanosoma gambiense*¹⁰. Thus, the residual immunity, however slight, seems to have as great a bearing on the outcome of infection as does the profound immunosuppression. This point may be relevant to the wide spectrum of infectious diseases for which immunodepression has been described.

A relationship between variable antigenic type and trypanosome virulence was first proposed by McNeillage and Herbert¹¹. In the present study, although clones NIM 2 and 6 are antigenic variants or subclones derived from a common strain, they differ slightly with respect to both virulence and immunosuppressive activity. The possibility that the variant antigens are themselves responsible for the suppression is now being investigated.

Apart from the intrinsic suppressor activities of *T. brucei* strains, the level of parasitaemia (suppressor dose) undoubtedly contributes to the overall pattern of secondary immunodeficiency. This and previous studies² have generally noted an inverse relationship between the survival time and parasite load during the first peak of parasitaemia. However, the level of initial parasitaemia may itself reflect the degree of suppression induced by trypanosome growth early in infection. This is only true for those clones which have comparable replication rates (for example, NIM 2, 6 and 7), which must therefore establish different levels of growth as a consequence of host immune parameters.

Antigenic variation seems to be the principal mechanism whereby the survival of African trypanosomes is prolonged in the host. Depression of anti-trypanosome responses might be required to prevent rapid, complete elimination of the parasite before antigenic variants are expressed. On the other hand, at least partial responsiveness is required to prevent one variant from growing in an uncontrolled fashion, a condition which would also preclude expression of future variants. If the goal is an overall, non-qualitative depression of responsiveness to a residual level, then IgG responses could be affected first because they are more susceptible to general mechanisms of suppression,

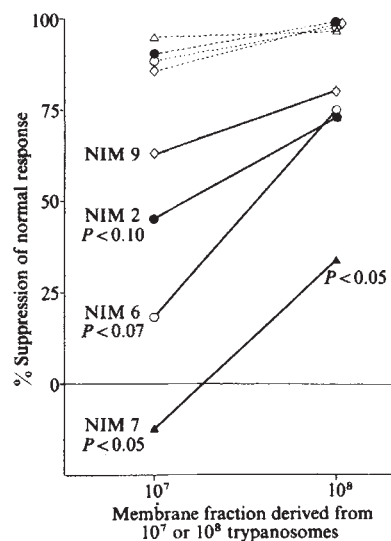


Fig. 2 Comparative suppression of SRBC response by membrane fractions derived from four clones of *T.b. brucei*. Crude membrane fractions from 10^8 or 10^7 trypanosomes were prepared as previously described⁸. Briefly, freeze-thawed homogenates of each clone were spun at 12,000g to remove nuclei, kinetoplasts and large debris. Membrane fractions were collected by high speed centrifugation (150,000g for 90 min), suspended in phosphate-buffered saline and injected intraperitoneally into (CBA/H×C57BL/6)_F₁ female mice. Mice were primed 4 d later with 4×10^7 SRBC, and the direct and indirect anti-SRBC response assayed 6 d after priming by the slide modification of the Jerne technique¹⁴. Each point is the arithmetic mean (five mice per group) of the per cent suppression of the normal response. —, Direct (IgM) PFC; ----, indirect PFC developed using a rabbit antiserum specific for mouse IgG. *P* values were ascertained by Student's *t*-test for comparison of each mean value with the corresponding next greater mean. Statistic is omitted when no significant difference between the respective mean values was found.

Table 1 Inhibition of immune response to SRBC after infection with different clones of *T. b. brucei*

Expt no.	Infecting clone	Direct PFC	% Normal	Indirect PFC	% Normal
1	—	53,000 ± 8,400		169,000 ± 25,000	
	NIM 6	1,200 ± 2,100*	2	<500	<1
	NIM 2	900 ± 1,300*	1	<500	<1
2	—	84,000 ± 10,400		143,000 ± 33,000	
	NIM 7	14,200 ± 5,100	17	600 ± 420	<1

Mice were infected with 200 parasites and primed with 4×10^7 SRBC 10 d later. Assay of direct PFC on day 4 and indirect PFC on day 6 were as in Fig. 2; values represent the arithmetic mean $\pm$ s.d.; four to five mice per group.

* Value not significantly different from background PFC of unprimed, infected mice.

including disturbances in lymphoid function at the level of B and T cells and macrophages. Alternatively, selective inhibition of IgG as opposed to IgM responses might be purposeful and advantageous to parasite survival. Terry has suggested that although IgM antibody is effective in the control of circulating trypanosomes, it is less effective than IgG classes in reaching tissue parasites from which antigenic variants are thought to arise¹. The present data are strikingly consistent with this interpretation. Complete suppression of IgG responses and only partial suppression of IgM seems to ensure chronicity of infection, and the intrinsic differences between *T. brucei* clones in suppressing IgM responses seem to form a basis for parasite virulence.

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Biochemical basis for the continuous copulation of female *Schistosoma mansoni*

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Sexual dimorphism in blood flukes of the family Schistosomidae is marked, and until the male clasps the female in the gynaecophoric canal the female cannot attain sexual maturity¹ or migrate out of the liver². When previously-paired egg-producing females are transferred to a new host without males, the vitelline cells necessary for egg production degenerate³. By means of sensitive fluorographic analysis of electrophoresed polypeptides, we have now found molecular evidence of an obligatory continuous relationship between the sexes in *Schistosoma mansoni*. The male worm retains little of the protein it produces in greatest abundance, and this protein is electrophoretically identical to the most abundant protein found in, but not synthesised by, the female.

To detect which proteins were actually produced by the worms, *S. mansoni* of the NIH strain were perfused from C57BL/6B mice at 70 days post-infection, mechanically separated according to sex, and incubated for 24 h in medium HB597 (Connaught) with 10% fetal calf serum and $8 \mu\text{Ci ml}^{-1}$ ^{14}C -leucine (Specific activity $339 \text{ mCi mmol}^{-1}$; NEN). The worms were washed in saline and homogenised in 2% SDS and 5% β -mercaptoethanol in 80 mM Tris-HCl (pH 6.8), heated at 100°C for 1 min, cooled and precipitated with 20% trichloroacetic acid (TCA). After centrifugation at $5,000 \text{ g}$ for 10 min, the pellets were washed twice with cold 5% TCA, washed in 5% TCA at 90°C for 15 min, washed with ethanol/ether (2:1) saturated with sodium acetate, washed in ether and dried overnight *in vacuo*. The dried protein precipitates were resolubilised in the homogenising solution, the protein determined by TCA-turbidity⁴ and radioactivity measured in a Beckman LS-255 liquid scintillation counter. Vertical SDS-polyacrylamide slab gels were prepared by the method of Laemmli⁵, and known amounts of radioactivity or protein were added to each well. Standards of known molecular weight (MW), mouse serum and proteins of the *in vitro* culture medium were co-electrophoresed. After photography of the Coomassie blue staining patterns, the gels were prepared for fluorography^{6,7}, wherein the dried gel containing isotope-labelled peptides is apposed to pre-flashed Kodak X-Omat R X-ray film and exposed at -70°C . Only newly synthesised polypeptides having incorporated radioactive leucine from the *in vitro* medium show up as bands in the fluorogram. The procedure was also carried out with a strain of *S. mansoni* inbred in the laboratory of R. B. Short (Florida State University), grown in a white mouse host. The results were virtually identical, with only very minor quantitative differences perceivable in both staining and fluorograms. Protein similarity among *S. mansoni* strains was also found immunoelectrophoretically by Capron *et al.*⁸.

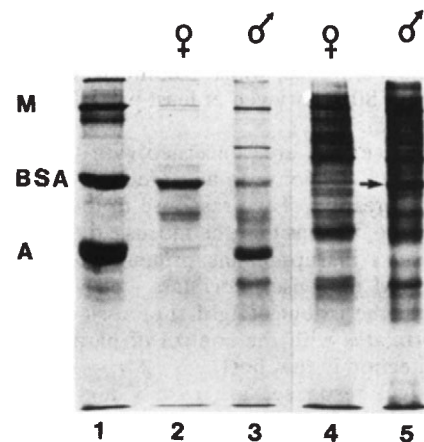


Fig. 1 SDS-8% polyacrylamide gel electrophoretic patterns of the polypeptides of adult male and female *Schistosoma mansoni*. In lane 1 of this composite photograph of a single gel, standard purified polypeptides are stained with Coomassie blue. M = myosin heavy chain, MW 200,000; α = α -actinin, 105,000 MW; BSA = bovine serum albumin, 58,000 MW; A = actin, 43,000 MW; T = tropomyosin, 31,000 MW. The stained patterns of the female (lane 2) and male (lane 3) reveal a high degree of identity, but a prominent band in the female is evident at about 66,000 MW. As equal amounts of protein were placed in these wells, this is evidently a striking quantitative difference in comparison with the faint band in the male. Lanes 4 and 5 are photographs of the fluorograms exposed by the same samples shown stained in lanes 2 and 3. Equal amounts of radioactivity were placed in the wells, and these autoradiographs show which proteins were synthesised by isolated worms *in vitro*. That the females do not produce their most prominent stained band is striking. The males, on the other hand, actively produce a polypeptide at this MW of which they have only small quantities, as seen stained in lane 3.

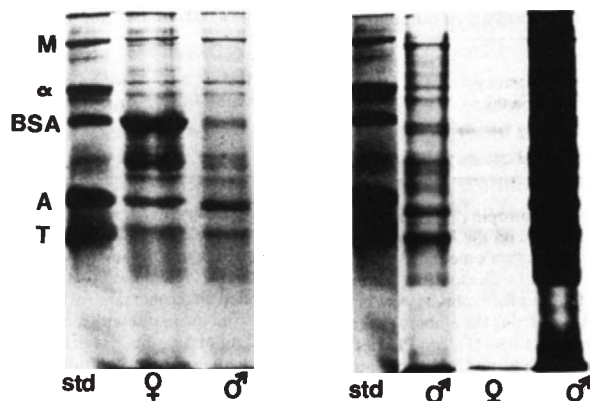


Fig. 2 SDS-10% polyacrylamide gel electrophoretic patterns of unlabelled female *S. mansoni* after remaining in copula with labelled males for 20 h. On the left are the Coomassie blue staining patterns of the samples shown in the three fluorograms of the composite picture on the right (the standards are a stained preparation). The male and female samples on the far right are a comparison exposure to show the low radioactivity profile of the females; the male fluorographic pattern next to the standard was prepared with a shorter exposure. The 66,000-MW protein is picked up by the female in greater amounts than any other. The other proteins supplied to the female by the male mirror his most actively synthesised proteins. Prolonged exposure of the female samples suggested a quantitative synthetic pattern typical of the male. (Abbreviations as in Fig. 1.)

Figure 1 is a composite of the Coomassie blue patterns and fluorograms of the total proteins of male and female *S. mansoni*. In the female, the heavily stained band with a mobility slightly faster than that of bovine serum albumin (BSA) is outstanding. Its absence in the fluorogram of the same gel means that this protein could not have been synthesised by the female. Two-dimensional electrophoretic analysis of this protein indicates that its MW and isoelectric point do not match any of the proteins found in the culture medium or the mouse serum, and so this does not represent a 'captured' protein.

The absence of a major protein synthesised uniquely by mature females was at first disappointing, as it was expected that the commitment to egg and vitelline cell production would be reflected in the proteins produced. However, quantitative differences in the amount of a protein produced by the male (arrow, Fig. 1) but found in relatively low amounts (revealed by the staining pattern) compared with the female pointed to the transport of this substance transtegumentally between the sexes. The fact that the female is almost entirely enveloped by the male tegument and that the male gynaecophoric canal has a modified surface⁹ supported this view.

To characterise further the nature of the 66,000-MW protein in question, the experiment was repeated with males labelled for 24 h, then rinsed and mated to unlabelled females for 20 h. Figure 2 depicts the fluorogram developed after electrophoresis of the polypeptides of the females following removal of the males and three washes with fresh medium. Transfer of the labelled polypeptide from the male to the female within 20 h was significant; the females also picked up some minor common polypeptides. No radioactivity was detectable in the TCA-soluble fractions from the homogenised females, indicating the absence of free ¹⁴C-leucine. The medium in which the mated worms had been incubated contained negligible amounts of radioactivity (<30 c.p.m. μ l⁻¹), so it is very unlikely after the thorough rinsing that the labelled polypeptides revealed in the fluorogram were a result of passive transfer. The concept of direct transtegumental transport between the sexes is supported by the studies of Shaw *et al.*¹⁰, who found that the vitelline cells of immature females were stimulated to develop by extracts of whole male worms, but not by the medium in which the males were cultured. It is unlikely that the protein is transferred with

sperm, as in butterflies¹¹, because the seminal receptacle is only about 0.1% of the female's volume, whereas the 66,000-MW band is about one-tenth of her total stained protein.

Our subsequent work with two-dimensional electrophoresis and fluorographic analysis (manuscript in preparation) has shown that this protein represents the major difference between the male and female in terms of their acid-precipitable proteins. Ruppel and Cioli¹² have published a polyacrylamide gel electrophoresis staining pattern with similar results, although they do not discuss this protein with a mobility slightly faster than BSA.

The absence of parthenogenesis in the genus *Schistosoma* (except for *Schistosoma mattheei*¹³) seems predictable in the light of this molecular symbiosis maintained by the sexes for the production of offspring. A cure for schistosomiasis may consist of finding a method of keeping the sexes unpaired.

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The *in vitro* transmission frequency of the *t⁶* allele

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The *T* region, located on chromosome 17 in the mouse, is a complex genetic locus which includes a series of recessive mutations, *tⁿ* (refs 1, 2). These mutations include several alleles (also referred to as *t*-haplotypes²) which are embryonic lethals (*t^L*). An additional phenotypic expression of the recessive lethal mutations is a distorted transmission of the *t* allele by the heterozygous male parent. Most *T/t^L* and *+/t^L* males transmit the recessive mutation to over 50% of their offspring. Two conditions are known to modify significantly the transmission frequency of some *t^L* alleles: the length of time between insemination and fertilisation³⁻⁵ and the genetic background of the female⁶. The present studies were undertaken to determine if the distorted transmission frequency of one of the lethal alleles, *t⁶*, could be reproduced *in vitro*. The data show that the *in vitro* transmission frequency of the *t⁶* mutation is non-mendelian and is not significantly different from the frequency of transmission of this mutation following *in vivo* delayed matings. The *in vitro* frequency is, however, significantly lower than the normal mating frequency and does not vary as a function of incubation time. These data suggest that the observed delayed and normal mating frequency differences result from time-dependent interactions between the *t⁶*-bearing spermatozoon and the female reproductive tract.

Table 1 Normal and delayed transmission frequencies of the t^6 allele determined by the *in vitro* culture of one-cell embryos fertilised *in vivo*

	Control cross $T/+ \times +/t^6 \delta$		Experimental cross $+/t^6 \delta \times +/t^6 \delta$	
	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype
Normal mating	409	0	724	187
Delayed mating	281	0	481	58
				Transmission frequency*
				0.52
				0.24

(C57BL/6J $\times T/t^6 \delta$) F_1 females were injected intraperitoneally with pregnant mare serum gonadotropin (5 IU) followed 48 h later by an injection of human chorionic gonadotropin (HCG; 5 IU)²⁵. The females were caged with $+/t^6$ [(BALB/c $\times T/t^6 \delta$) F_1] males and on the following day the pregnant females were killed by cervical dislocation. One-cell embryos were flushed from the extirpated oviducts and placed in modified Whitten's medium^{8,26} (4 mg ml⁻¹ bovine serum albumin, Miles; 75 μ M Na₂EDTA) under silicone oil (Dow Corning 200 Fluid, 50 cSt viscosity). The silicone oil (equilibrated with modified Whitten's medium, 7:1 v/v) and modified Whitten's medium were gassed with 5% O₂, 5% CO₂ and 90% N₂ before use. For the first two cleavage divisions the embryos were cultured in a sealed anaerobic jar (Torbal) which had been gassed for 20 min with 5% O₂, 5% CO₂ and 90% N₂. At the four-cell stage the dishes containing the embryos were transferred to an incubator with a continuous gas flow of 5% CO₂ in air. Blastocyst-stage embryos were transferred to a 35 $\times$ 10 mm tissue culture dish (Falcon Plastics) containing 2 ml of modified Eagle's medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum (GIBCO)⁹. The dishes were returned to the continuous gas flow incubator for an additional 4 days. Outgrowths were scored as mutant or non-mutant using a Zeiss inverted microscope equipped with phase contrast optics ($\times$ 160) on days 8 and 9 post-fertilisation. Preimplantation development at normal mating times from the two-cell stage to the blastocyst stage for the control and experimental crosses was 456/486 (94%) and 814/872 (93%), respectively, and at delayed mating times 311/321 (97%) and 531/546 (97%), respectively. Outgrowth of the blastocysts at normal mating times for the control and experimental crosses was 409/447 (91%) and 724/798 (91%), respectively, and delayed mating times 281/310 (91%) and 481/528 (91%), respectively. Significant differences were computed using a χ^2 contingency test. For normal matings, females were caged with males immediately after the HCG injection. For delayed mating, females were caged with males 12 h after the HCG injection.

* Transmission frequency = $2 \times (\text{no. of } t^6/t^6 \text{ outgrowths} / \text{total no. of outgrowths})$.

These studies were possible because: (1) ova from F_1 hybrid females fertilised either *in vitro*⁷ or *in vivo*⁸ can develop to the blastocyst stage when incubated in modified Krebs-Ringer medium; (2) these blastocysts will continue to develop ('out-grow') when placed in modified Eagle's medium⁹; (3) the outgrowths from t^6/t^6 uterine blastocyst embryos can be distinguished morphologically from their phenotypically wild-type littermates¹⁰⁻¹²; (4) the number of mutant outgrowths in these genetically mixed embryo populations is equivalent to the expected number based on the tested transmission frequency of the t^6 allele¹¹. Mutant outgrowths developing *in vitro* from uterine blastocysts can be distinguished from their normal counterparts by the failure of their trophoblast cells to polyploidise and become giant and by a reduced viability of the inner cell mass cells. However, only the former criterion could be used in the present studies because outgrowths developing from ova fertilised *in vitro* may not contain an inner cell mass.

To ensure the development of embryos in culture following *in*

vitro fertilisation, ova from hybrid females were used^{13,14}. These females were obtained by crossing C57BL/6J females with T/t^6 males. The offspring from this cross are phenotypically distinguishable: $+/t^6$ females have tails of normal length whereas $T/+$ females are short-tailed. Ova from the $T/+$ females were used as the controls for the *in vitro* fertilisation studies.

Before using spermatozoa from $+/t^6$ males for *in vitro* inseminations, we tested the males for their transmission frequencies in normal and delayed matings with the hybrid $+/t^6$ females. Following normal matings, spermatozoa reside in the uterus for 6-7 h before ovulation and fertilisation. In delayed matings, fertilisation is initiated 1-2 h post-insemination¹⁵. After both normal and delayed matings, the fertilised one-cell embryos were removed from the female and placed in culture. Blastocyst outgrowths were scored to distinguish the t^6 homozygotes. Table 1 gives the percentage transmission of the t^6 allele from $+/t^6$ males following both normal and delayed matings with hybrid $+/t^6$ females. The data show that in normal

Table 2 *In vitro* transmission frequency of the t^6 allele

Male no.	<i>In vivo</i> *		<i>In vitro</i>				
	Transmission frequency†		Control cross $T/+ \times +/t^6 \delta$		Experimental cross $+/t^6 \delta \times +/t^6 \delta$		Transmission frequency†
	Normal mating	Delayed mating	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	
1	0.70 (43)‡	—	21	0	96	12	0.25
2	0.52 (66)	—	108	0	87	6	0.14
3	0.41 (58)	—	68	0	108	14	0.26
4	—	0.16 (49)	90	0	78	8	0.21
5	—	0.18 (46)	37	0	68	6	0.18
6	—	0.38 (47)	43	0	56	8	0.29
			Total 367	0	493	54	0.22

(C57BL/6J $\times T/t^6 \delta$) F_1 females were injected as described in Table 1 legend. At 12-13 h after the HCG injection the females were killed and their oviducts excised and placed in drops of modified Tyrode's medium²⁷ under silicone oil. The cumulus masses containing the ova were removed from the ampullary regions of the oviducts and twice transferred to fresh drops of medium (0.2 ml) under silicone oil. Spermatozoa were obtained from the cauda epididymides and vasa deferentia excised from (BALB/c $\times T/t^6 \delta$) F_1 males. The ducts, placed into 1 ml of modified Tyrode's medium under silicone oil, were minced and the spermatozoa allowed to disperse for 20 min. A 100- μ l sample of the spermatozoan suspension was diluted approximately 1:3 with modified Tyrode's medium. A 10- μ l aliquot of the diluted suspension was added to the 0.2-ml drops of medium containing the ova. Final spermatozoan concentrations ranged from 1.3 to 2.7×10^5 spermatozoa per ml. The insemination dishes were placed in an anaerobic jar and gassed for 20 min with 5% O₂, 5% CO₂ and 90% N₂ at 37 °C. The jar was subsequently sealed and the gametes were allowed to co-incubate for 6 h. After this 6-h period, the ova were removed and washed through four drops of modified Whitten's medium. Preparation of the fertilisation medium, culture medium, silicone oil and the culture of the embryos were as described in Table 1 legend. The procedures for *in vitro* fertilisation followed the protocol of Fraser and Drury²⁷ except that the sodium chloride concentration in the modified Tyrode's medium was reduced to 85.2 mM. Fertilisation, based on the number of one-cell ova that cleaved to the two-cell stage, averaged 86% (75-96% range). Preimplantation development from the two-cell stage to the blastocyst stage for the control and experimental crosses was 446/511 (87%) and 565/653 (87%), respectively. The number of blastocyst outgrowths/total number of blastocysts from the control and experimental crosses was 367/417 (88%) and 493/554 (89%), respectively.

* Individual males, before being used for the *in vitro* fertilisation experiments, were tested *in vivo* for their transmission frequencies at either normal or delayed mating times. The times at which the animals were mated and the subsequent culture of the one-cell embryos were as described in Table 1 legend.

† Transmission frequency = $2 \times (\text{no. of } t^6/t^6 \text{ outgrowths} / \text{total no. of outgrowths})$.

‡ Number in parentheses refers to the number of outgrowths on which the *in vivo* transmission frequency value is based.

Table 3 Effect of *in vitro* incubation period on *in vitro* transmission frequency of the t^6 allele

Male no.	<i>In vivo</i> *		<i>In vitro</i>				
	Transmission frequency†		0 h No. of outgrowths with t^6/t^6 phenotype	Transmission frequency†	6 h No. of outgrowths with t^6/t^6 phenotype	Transmission frequency†	
	Normal mating	Delayed mating	No. of blastocyst outgrowths		No. of blastocyst outgrowths		
7	0.38 (52)‡	0.00 (29)	87	0.16	89	0.18	
8	—	—	100	0.14	43	0.19	
9	0.60 (73)	0.28 (36)	72	0.17	41	0.24	
10	0.40 (45)	0.09 (45)	58	0.31	75	0.11	
			Total 317	0.18	248	0.17	

Females were injected so that recently ovulated oocytes were available at 0 and 6 h. The gametes were obtained from (C57BL/6J $\times$ T/ t^6)F₁ females and (BALB/c $\times$ T/ t^6)F₁ males as described in Table 2 legend. The 0-h data list the results from inseminations with diluted spermatozoan suspensions mixed with ova immediately after the 20-min dispersal period. During the 6-h *in vitro* incubation period the spermatozoa were maintained in 1 ml of modified Tyrode's medium at 37 °C in a gas phase of 5% O₂, 5% CO₂ and 90% N₂. An aliquot of the spermatozoan suspension was then freshly diluted and added to the second group of oocytes. Spermatozoan concentrations ranged from 2.1 to 4.3 $\times$ 10⁵ spermatozoa per ml.

* Individual males, before being used for the *in vitro* fertilisation experiments, were tested *in vivo* for their transmission frequencies at either normal or delayed mating times. The times at which the animals were mated and the subsequent culture of the one-cell embryos were as described in Table 1 legend.

† Transmission frequency = 2 $\times$ (no. of t^6/t^6 outgrowths/total no. of outgrowths).

‡ Number in parentheses refers to the number of outgrowths on which the *in vivo* transmission frequency value is based.

matings the transmission frequency of the t^6 allele is mendelian (0.52) and that the transmission frequency of the t^6 allele is significantly reduced (0.24) if the matings are delayed until the time of ovulation ($\chi^2 = 33.0$; $P < 0.0001$). These findings support not only the earlier observation that the length of time between insemination and fertilisation affects the transmission frequency of the t^6 allele¹⁶, but also the observation that the genotype of the female can modify the transmission frequency of certain t^L alleles. Males which are heterozygous for the t^6 allele typically transmit the allele at a frequency of greater than 70% in matings with BALB/c (ref. 17) and SWR/J (ref. 11) mice. This is significantly higher than the frequency of transmission of this allele in matings with either the hybrid females or C57BL/6J females. In the latter matings (T/ t^6 $\times$ C57BL/6J), the transmission frequency (0.46) was determined from the number of normal-tailed offspring among the total number of progeny (1,070/2,333).

For the *in vitro* fertilisation studies, ova from hybrid T/+ and +/ t^6 females were fertilised *in vitro* by spermatozoa from +/ t^6 males. Aliquots of spermatozoa from the same male were used to fertilise oviducal ova from females of both genotypes. The results (Table 2) show that all the 367 outgrowths developing from fertilised T/+ ova developed normally and all exhibited the wild-type phenotype. Of the ova obtained from +/ t^6 females and fertilised *in vitro*, 54 of the 493 outgrowths were phenotypically abnormal and were scored as homozygous mutant embryos. Thus, the transmission frequency of the t^6 allele *in vitro* is 0.22. This frequency is significantly less than the frequency observed during *in vivo* normal matings ($\chi^2 = 39.9$; $P < 0.0001$). Conversely, the difference between the *in vitro* transmission frequency and the *in vivo* delayed frequency is not significant ($\chi^2 = 0.19$; $P > 0.05$).

In vitro, fertilisation begins 1–2 h after insemination^{18–20}. During this period, the spermatozoa undergo capacitation and penetrate the investments surrounding the ovum. In delayed matings the spermatozoa also reside in the female reproductive tract, before fertilisation, for 1–2 h (ref. 15). In normal matings, however, the time between insemination and fertilisation is considerably longer.

Because *in vitro* insemination and *in vivo* delayed insemination result in similar reductions in the transmission frequency of the t^6 allele, and because both procedures are associated with reduced time between insemination and fertilisation, we tested the affect of 'time *in vitro*' on the frequency of t^6 -allele transmission. We compared the transmission frequency of this allele by using aliquots of gametes from the same male to inseminate ova following either a 0 or 6 h incubation period of the sper-

matozoa. The results (Table 3) show that the increased period of incubation does not increase the *in vitro* transmission frequency of the t^6 allele (0.18 versus 0.17; $\chi^2 = 0.018$; $P > 0.05$).

The data from these latter studies disprove the hypotheses that the distorted transmission frequency of a t -allele is caused by either a selective death of the non- t -bearing spermatozoa with time^{21,22} or a differential transport of the + and t -bearing gametes through the uterotubal junction^{23,24}. Our findings suggest, rather, that the increase in transmission frequencies between delayed and normal matings is dependent on an interaction between the female reproductive tract and the male gamete, and that this interaction is time dependent. As the transmission frequency of the tested allele can be significantly affected by the genotype of the female, it is probable that the enhancing factor is intrinsic to the uterine and oviducal environments and is variable among the females of different genotypes.

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Evidence for a role of plasma kallikrein in the activation of prorenin

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The proteolytic enzyme renin seems to play an important part in the humoral control of blood pressure by initiating a reaction which finally leads to the production of the vasoconstrictor peptide angiotensin II. However, it has recently been shown that normal human plasma contains a higher molecular weight form of renin (prorenin), which is enzymatically inactive¹⁻⁶. *In vitro*, prorenin is converted to the active renin enzyme by various unphysiological procedures such as cryoactivation⁷⁻⁹, acid activation^{1,6,7,9} or the addition of trypsin^{10,11} or pepsin^{11,12}, the activation by trypsin being equal to that by acidification¹³⁻¹⁵. Although the physiological role of prorenin remains unclear, it has been shown that differences exist in the response to stimuli between prorenin and the active renin enzyme^{3,6,7}. Recently, it has been shown that serine protease(s) may be involved in the activation process of prorenin, during both cryoactivation^{7,16} and acid activation⁷. Furthermore, human urinary kallikrein added to plasma in low concentrations is able to activate prorenin completely *in vitro*¹⁷. Therefore, it has been speculated that urinary kallikrein might be the physiological *in vivo* activator of prorenin¹⁷⁻¹⁹. This also seemed plausible because renal kallikrein is produced in cells adjacent to the renin-producing cells²⁰. On the other hand, plasma kallikrein has been implicated in the activation of prorenin^{15,21-23}. In the experiments described below we tried to obtain some more direct evidence for the *in vivo* role of kallikrein(s) in the prorenin activation. Our results suggest that plasma kallikrein may indeed be a physiological activator of prorenin, but they do not support a role for urinary (renal) kallikrein in the activation of prorenin.

If kallikrein is the *in vivo* activator of prorenin, a negative correlation between kallikrein (urinary or plasma) and the proportion of prorenin (prorenin/total renin) may be expected. In 13 normal human volunteers, urinary kallikrein excretion and plasma prekallikrein concentration were correlated to the proportion of prorenin in plasma in different states of sodium balance, which is known to have a marked effect on urinary kallikrein excretion. Urinary kallikrein²⁴ and plasma prekallikrein^{24,25} were measured using chromogenic tripeptide substrates (substrates S2266 and S2302, Kabi Diagnostica). Active renin and prorenin were determined by radioimmunoassay of angiotensin I using acid activation and nephrectomised sheep plasma as renin substrate⁶.

Whereas sodium excretion was significantly reduced during sodium restriction (diet containing approximately 1.0 g NaCl per day) and, concomitantly, urinary kallikrein excretion

increased fourfold, plasma prekallikrein concentration and the proportion of prorenin remained essentially unchanged (Table 1). During sodium loading (*ad libitum* diet plus 7.5 g NaCl per day for 3 days) sodium excretion was significantly higher than during the control period. Urinary kallikrein excretion was still elevated, presumably because of a significantly elevated urinary volume. Plasma prekallikrein and the proportion of prorenin did not change significantly during sodium loading.

Figure 1 shows that during all experimental periods, there was no correlation between urinary kallikrein excretion and the proportion of prorenin. This is in contrast to the results of other authors, who found a weak correlation between these two parameters¹⁹. In our experiments, in spite of profound alterations in urinary kallikrein excretion, the proportion of prorenin remained unchanged. This certainly does not exclude a role of urinary kallikrein in the activation of prorenin, but does make it rather improbable.

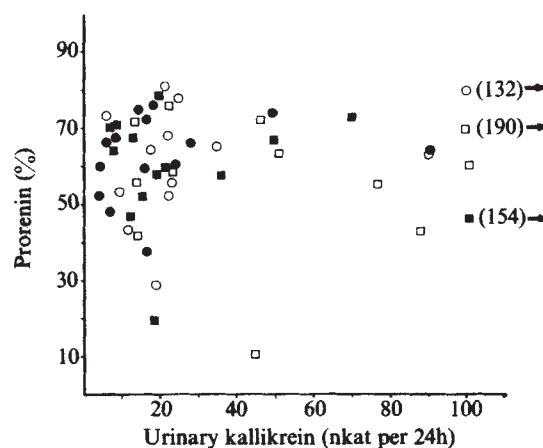


Fig. 1 Urinary kallikrein excretion and proportion of prorenin in different states of sodium balance in 13 normal human volunteers. ●, Normal diet; ○, sodium loading; ■, sodium restriction (2 days); □, sodium restriction (4 days).

Although during the different experimental periods plasma prekallikrein did not change significantly (Table 1), a close relationship between plasma prekallikrein concentration and the proportion of prorenin (Fig. 2) was observed: the higher the plasma prekallikrein, the lower the proportion of prorenin in plasma. The linear correlation coefficients were significant during all periods of the experimental protocol (*ad libitum* diet: $y = 96.8 - 0.64x$; $r = 0.71$; $n = 13$; $P < 0.01$. Sodium restriction: $y = 102.9 - 0.85x$; $r = 0.70$; $n = 22$; $P < 0.001$. Sodium loading: $y = 97.5 - 0.69x$; $r = 0.60$; $n = 13$; $P < 0.05$).

There are two possible explanations for the correlation. First, that this is an *in vitro* effect, which is due to incomplete activation of prorenin by the acidification procedure. However, this seems

Table 1 Urinary volume, sodium excretion, urinary kallikrein excretion, plasma prekallikrein and proportion of prorenin in different states of sodium balance

	<i>ad libitum</i> diet	1	Days of sodium restriction			Sodium loading
			2	3	4	
Urinary volume (ml per 24 h)	1,316 ± 143	1,551 ± 183	1,418 ± 175	1,343 ± 176	1,338 ± 113	1,916 ± 264†
Sodium excretion (mmol per 24 h)	170.0 ± 19.8	124.8 ± 14.8‡	48.9 ± 4.8§	27.7 ± 4.1†	16.4 ± 1.9§	331.7 ± 31.3§
Urinary kallikrein excretion (nkat per 24 h)	16.4 ± 3.4	21.3 ± 4.4‡	22.9 ± 5.1†	50.8 ± 12.1‡	64.6 ± 15.6‡	33.2 ± 10.0*
Plasma prekallikrein (μkat l ⁻¹)	53.2 ± 3.6		48.6 ± 3.6		52.3 ± 5.0	51.4 ± 3.6
Proportion of prorenin (%)	62.7 ± 3.2		60.5 ± 4.2		57.1 ± 4.7	61.0 ± 4.5

Values are given as means ± s.e.m., $n = 13$. Significantly different from controls on an *ad libitum* diet: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.005$; § $P < 0.001$.

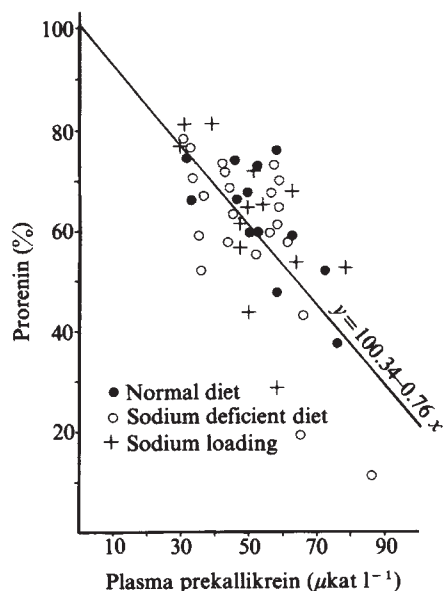


Fig. 2 Correlation between plasma prekallikrein and the proportion of prorenin in 13 normal human volunteers in different states of sodium balance. $r = 0.6652$; $n = 48$; $P < 0.001$.

improbable as it has been repeatedly shown that the so-called 'acid'-activation is complete and equivalent to the activation by trypsin¹³⁻¹⁵. Second, the correlation found may be physiologically significant and is applicable to the *in vivo* situation. This, of course, would be a strong argument in favour of an *in vivo* role of plasma kallikrein in the activation of prorenin.

Interestingly, the intercept on the ordinate in all three groups was near 100%, suggesting that in a plasma free of prekallikrein, renin would only be present in the form of prorenin and active renin enzyme would not be formed in these conditions. This, however, would suggest that plasma kallikrein not only is one of several activators of prorenin, but also that it may be the only activator of physiological significance. However, as our results were obtained in normal subjects only, we cannot exclude the possibility that in certain pathological conditions the relationship demonstrated may not apply.

Thus, our results do not support the concept that urinary kallikrein is of importance in the *in vivo* activation of prorenin, as has been proposed¹⁷⁻¹⁹, but they strengthen the view that plasma kallikrein might be the physiological activator of prorenin^{15,21,22}.

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Multiple innervation of cerebellar Purkinje cells by climbing fibres in *staggerer* mutant mouse

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Development of the peripheral nervous system involves an initial overgrowth of synaptic contacts, followed later by the regression of the redundant connections¹⁻⁴. Similarly, in the central nervous system, the adult-type one-to-one relationship between climbing fibres (CFs) and cerebellar Purkinje cells (PCs) is preceded at early developmental stages by a multiple innervation of PCs by CFs⁵. The regression of the supernumerary contacts fails to occur when PCs develop in a cellular environment devoid of granule cells⁶⁻⁹, suggesting that it may depend on the formation of synapses between parallel fibres and PCs. However, one cannot preclude the possibility that granule cells intervene in the regressive process by a nonsynaptic action, for instance by emitting a chemical signal at the level of their soma, which could act on the CF-PC synapses. In this respect, the recessive *staggerer* (*sg*) mutation¹⁰, is of special interest because one of its major phenotypical expressions in homozygous *sg/sg* animals is the selective and almost complete absence of synapse formation between parallel fibres and Purkinje cells, in spite of the presence of an initially important number of granule cells and of parallel fibres during the synaptogenesis period, that is, until about the end of the 3rd post-natal week. Later on, the relay cells progressively die, probably because of the absence of synaptic contacts with PCs¹¹⁻¹³. This is in marked contrast with the other agranular animals studied so far (X-ray irradiated rats and *weaver* mutant mice^{6,7,9}) where granule cells lack from the very beginning^{6,9,12}. As the *staggerer* mutation does not prevent the formation of functional synapses between CFs and PCs¹⁴, it was important to establish whether PCs can reach the adult-type monoinnervation in the mutant. We show here that each PC is contacted by several CFs in the adult homozygous *staggerer* mouse, thus supporting the first hypothesis.

Hybrids of C57BL and DBA2J mice originating from the Jackson Laboratory and bearing the mutation were raised at the Laboratory and homozygous mutants *sg/sg* were obtained by intercrossing heterozygous animals. Mutant mice were distinguished from their littermates by clinical features¹² and they were kept alive after weaning by the use of appropriate feeding conditions¹⁵. The organisation of the cerebellar cortex at the ultrastructural level in adult *sg/sg* animals was identical to that previously described (C. Sotelo, personal communication) and they were used for electrophysiological studies 5 to 7 months after birth. After initial ether or Nembutal (30 mg per kg intraperitoneally) anaesthesia, animals were paralysed,

artificially respired, and the vermis was exposed. Juxtafastigial (JF) stimulation was performed with fine bipolar electrodes and stimulation sites were confirmed as previously reported¹⁴. Intracellular recordings from PCs were obtained with glass micropipettes (d.c. resistances between 10 and 15 M Ω). An active bridge circuit was used to pass current through the recording microelectrode. The position of the tip of the microelectrode in the cerebellar cortex was determined by iontophoretic injection of methyl blue.

Twenty four PCs identified before cell penetration by their antidromic activation^{14,16} elicited by JF stimulation (data not shown) were impaled and their electrical activities were analysed. In all the recorded cells, the orthodromic responses evoked by JF stimulation closely resembled a CF response¹⁶: they consisted of an initial full spike followed by a prolonged plateau of depolarisation on which partial spikes were or not superimposed (Fig. 1ai). Cell deterioration led rapidly to a suppression of spike potentials within the response and the underlying excitatory postsynaptic potential (e.p.s.p.) was revealed (Fig. 1a_{ii}). It had the typical time course of a CF-e.p.s.p.¹⁶ but it was graded by steps (2 to 4) when increasing stimulation strength as it is usually observed in multiply innervated PCs^{6,9} (Fig. 1a_{iii}). This type of CF-like e.p.s.p. also occurred spontaneously and it fluctuated in size in a stepwise manner (Fig. 1a_{iii}), with a firing rate for each component of the response in the range of CF-mediated e.p.s.ps (1–2 s⁻¹). Moreover, harmaline injection (50 mg per kg intraperitoneally) induced a rhythmic bursting (5 to 10 s⁻¹) of these compound e.p.s.ps (Fig. 1bi, ii), confirming they were mediated via CF¹⁷. Finally, these responses were reversed by depolarising currents injected through the microelectrode (Fig. 2), as expected for chemically mediated synaptic potentials¹⁸, and current values for reversal were very similar (10–15 nA) to those found for normal CF-e.p.s.ps¹⁹ and for graded CF-e.p.s.ps in adult X-ray irradiated rats⁹.

It seems unlikely that these graded CF-like activities could be mediated through the mossy fibre-granule cell pathway since at the age studied, almost all granule cells have degenerated in the mutant and since maximum stimulation of the residual parallel fibres either evoked no response or evoked only a single spike in the PCs tested (unpublished observations). The rare parallel fibre-PC synapses which still exist in the mutant¹³ could nevertheless be responsible for the late and small e.p.s.ps (as

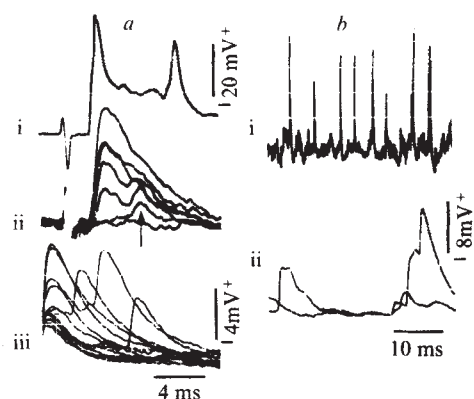


Fig. 1 Intracellular recordings from Purkinje cells. Body temperature was kept constant at 37 °C and the cerebellar vermis was covered with 4% agar in 10% sucrose solution. Resting potentials were -45 mV for the cell illustrated in *a*, and -35 mV for that in *b*. Cells generally remained in a good condition during intracellular recordings for up to 10 min. *ai*, Climbing fibre-like response evoked by JF stimulation; *ii*, same cell as in *ai* after cell deterioration. The underlying e.p.s.p. was graded in a stepwise manner with the intensity of the JF stimulation. *a_{iii}*, Spontaneous climbing fibre-like e.p.s.ps recorded from the same cell as in *ai* and *ii*. Each sweep was triggered by the rising edge of the response. *bi*, Rhythmic bursting of climbing fibre-like e.p.s.ps at about 5 s⁻¹ in another PC after harmaline injection; *b_{ii}*, same cell as in *bi* but with a faster sweep speed.

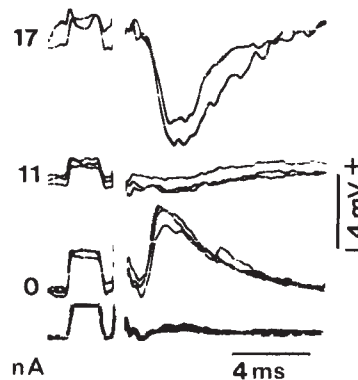


Fig. 2 Reversal properties of a climbing fibre-like e.p.s.p. evoked in a PC by JF stimulation under depolarising d.c. currents. The resting potential of the cell was -42 mV. Current injections were done when the spike generating mechanism of the cell was completely abolished. Ten suprathreshold JF stimulations at 0.5 s⁻¹ were applied for each current step (current values are indicated on the left of each record), and the size and polarity of the responses were determined by comparison with the extracellular field potential after withdrawal of the microelectrode (bottom trace). The calibrating pulse on the left of each trace is 2 mV in amplitude and 2 ms in duration.

compared to the CF-like response) which were sometimes evoked by JF stimulation in the recorded cells (Fig. 1a_{ii}, arrow) and which spontaneously occurred at a high firing rate (20–50 s⁻¹) during the resting discharge of PCs, thus corresponding to the simple spike spontaneous firing recorded before cell penetration.

The present study fully confirms the functional linkage between CFs and PCs in the *staggerer* mouse and establishes that each PC receives several independent CFs in the adult. These results allow the extracellular graded responses evoked in 3-week-old animals by JF stimulation¹⁴ and rhythmically triggered by harmaline injection to be now tentatively re-interpreted as immature CF-responses^{20,21} recorded from multiply innervated PCs whose maturation was very delayed²². At this early stage a significant number of granule cells is still present and the most striking abnormality is a selective absence of synapses between parallel fibres and PCs¹³. It therefore seems likely that in normal animals, the formation of parallel fibre-PC synapses is responsible for the regression of supernumerary CF-PC synapses which occurs during development. The present study strengthens the view that the *staggerer* mutation does not simply consist of a general reduction in the ability of PCs to form synapses with their afferents (as PCs are multiply innervated by CF) but that the loss of synaptic competence is highly selective.

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Imidazole inhibits a temperature-dependent component of mammalian skeletal muscle action potential

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Temperature-dependent transition of conductance in ionic channels has been described in several membrane systems¹⁻³. We report here an abrupt change in the maximal rate of rise of the action potentials of the rat extensor digitorum longus (EDL) at 32 °C, indicating alteration in the functional characteristics of the sodium channels. Tetrodotoxin (TTX) and imidazole were used to investigate further the change in properties of the sodium channels with respect to temperature. TTX is known for its specificity in blocking sodium channels⁴. It has been proposed that the guanidinium group of the TTX molecule is essential for its activity. Other guanidinium compounds have also been shown to be effective in blocking the sodium channels⁵. In this study, imidazole, a compound structurally similar to guanidine, was also tested. We find that TTX and imidazole affect the maximal rate of rise of the action potential in a temperature-dependent manner.

Extensor digitorum longus muscles from male Wistar rats were mounted in a muscle bath and perfused with oxygenated Ringer's solution. The temperature of the bath was regulated by water circulating around its jacket. Intracellular recordings were made with microelectrodes filled with 3 M KCl. The muscles were electrically stimulated with a pair of external platinum wire electrodes. The voltage change was recorded through a WPM4A electrometer and the maximal rate of rise of the action potential was obtained by a differentiating circuit. The maximal rate of rise of the action potential was measured because it gives an indication of the sodium conductance increase during an action potential⁶. The muscles were allowed to soak in the solutions containing the drugs for at least 20 min. before recording. At least 30 min was allowed for equilibration for any change of temperature.

The change in temperature, as well as TTX and imidazole, had no effect on the resting membrane potential of the muscle fibres. However, a dramatic change in the maximal rate of rise of the action potential with temperature was observed (Fig. 1). From a steady level of about 200 V s⁻¹ at temperatures below 32 °C, the rate increased to about double its value within a 5 °C temperature rise.

To investigate the effect of imidazole and TTX on the maximal rate of rise of the action potential with respect to temperature change, three representative temperatures (25 °C, 30 °C and 35 °C) were chosen for more detailed studies, although results obtained at other temperatures followed the same pattern. Imidazole reduced the rate of rise in a temperature-dependent manner. Within the lower temperature range from 20 to 30 °C, in which the maximal rate of rise did not change with temperature, imidazole had no effect. At temperatures above 32 °C, 2.5 × 10⁻⁵ M imidazole reduced the rate of rise to the same level as that at lower temperatures (Fig. 2c). Higher concentrations of imidazole (up to 10⁻³ M) were not effective in further reducing the rate of rise beyond that observed at lower temperatures.

TTX reduced the maximal rate of rise of the action potential at all temperatures (Fig. 2b). At 3.1 × 10⁻⁹ M TTX, the depression of the rate of rise was more conspicuous at 35 °C. It was only

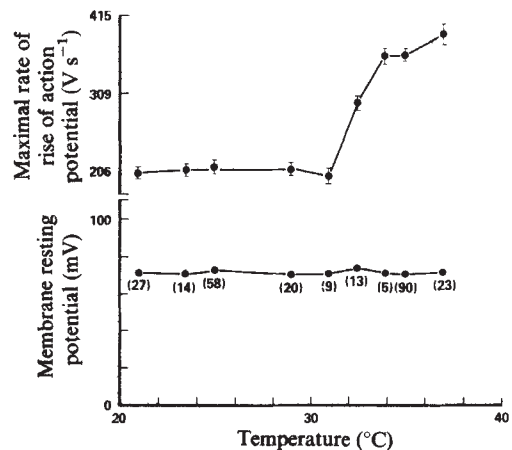


Fig. 1 Effect of temperature change on the maximal rate of rise of the action potential (top) and the resting membrane potential (bottom) of the rat extensor digitorum longus. Although the resting membrane potential was not sensitive to the variation in temperature, the rate of rise became temperature sensitive at around 32 °C, showing a sharp increase with only a small rise in temperature.

at higher concentrations of TTX (1.55 × 10⁻⁷ M) that the rate was reduced to the same level at all temperatures. Pretreatment of the muscle with imidazole (2.5 × 10⁻⁵ M) abolished the temperature sensitivity of the rate of rise to TTX. The rate was reduced to the same level at all temperatures (Fig. 2d). Increasing the TTX concentration resulted only in a concomitant reduction at the different temperatures.

The transition of the maximal rate of rise of the action potential at 32 °C indicates a sharp change in the current through the sodium channels of the muscle membrane. Change in membrane fluidity or conformational change of the channel protein has previously been proposed as the underlying cause for the change in properties of other ionic channels¹⁻³. It is also

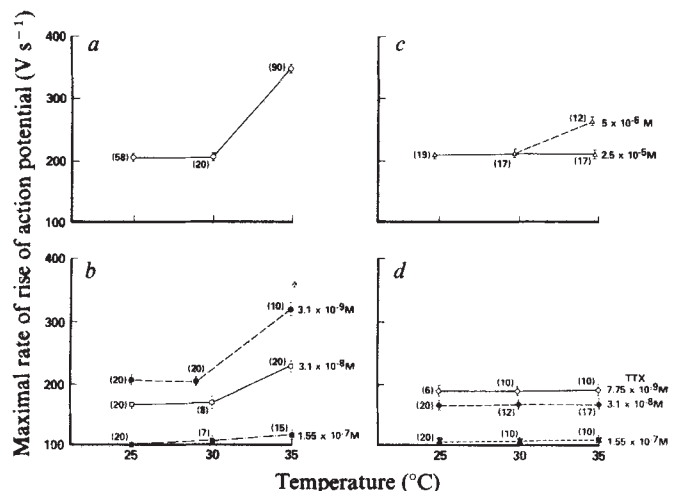


Fig. 2 Effect of imidazole and TTX on the maximal rate of rise of the action potential of the rat extensor digitorum longus at different temperatures. a, Control; b, effect of TTX. At a low TTX concentration (3.1 × 10⁻⁹ M) only the rate at 35 °C showed a significant reduction. At 3.1 × 10⁻⁸ M there was in addition a parallel shift of the rate between 25 and 30 °C. At 1.55 × 10⁻⁷ M, the difference in rate at different temperatures was completely eliminated. c, Effect of imidazole. Note that imidazole was effective in reducing the rate of rise only at 35 °C. The inhibition by imidazole was limited to the level as the rate of rise at low temperature. d, Effect of TTX after pretreatment with imidazole (10⁻⁴ M). Imidazole at 10⁻⁴ M abolished the difference in the rate of rise with respect to temperature. The effect of TTX in such conditions was to produce an inhibition that was not sensitive to temperature.

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possible that there is a separate population of temperature-sensitive channels.

The finding that imidazole only exerts its action at temperatures above 32 °C and reduces the rate of rise to that of the lower temperatures is difficult to explain. Whether imidazole can prevent changes in membrane fluidity or protein conformation has yet to be determined. Alternatively, the results may be interpreted to indicate two components contributing to the maximal rate of rise of the action potential: a temperature-independent component and a temperature-dependent component selectively blocked by imidazole. The latter is also more sensitive to TTX. In such a case, the action of imidazole may be due to its molecular structure, so that it selectively blocks the sodium channels activated at higher temperatures. If this were the correct interpretation, these results then implicate the existence of two types of sodium channel that are pharmacologically distinguishable using imidazole. The possibility that thromboxanes or the adenylate cyclase system may be involved in the regulation of the sodium current may also have to be considered as imidazole is a potent inhibitor of thromboxane synthesis⁷ as well as an activator of adenyl cyclase.

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Different components of black widow spider venom mediate transmitter release at vertebrate and lobster neuromuscular junctions

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Crude black widow spider venom (BWSV) has profound physiological effects on several neuromuscular preparations, both vertebrate and invertebrate¹. At frog and mouse neuromuscular junctions (NMJs), BWSV causes a massive increase in the frequency of miniature endplate potentials (m.e.p.ps)^{2,3} followed by a reduction in m.e.p.p. frequency and depletion of synaptic vesicles^{3–5}. Qualitatively similar physiological and morphological effects are also observed at lobster^{6–8} and insect^{9,10} NMJs after treatment with BWSV. Apparently, therefore, this venom can cause the release of several transmitters—acetylcholine at vertebrate NMJs and γ -aminobutyric acid (GABA) and glutamate at invertebrate NMJs¹¹. BWSV has also been shown to cause the release of acetylcholine, noradrenaline and GABA from slices of mouse cerebral cortex^{12,13}. α -Latrotoxin, a protein of molecular weight (MW) 130,000, had previously been shown to be responsible for the venom effects at vertebrate NMJs and in mouse brain slices^{12–14}. That finding prompted the question of whether α -latrotoxin was also responsible for transmitter release in the lobster preparation. The present report demonstrates that although the electrophysiological effects of BWSV on lobster and frog NMJs are similar, they are caused by different components of the venom. The effects on the lobster are attributable to fraction E, which contains a major 65,000-MW protein and several lower molecular weight species. It has previously been shown that fraction E causes firing of the crayfish stretch receptor¹⁴. Some of these results have been published elsewhere¹⁵.

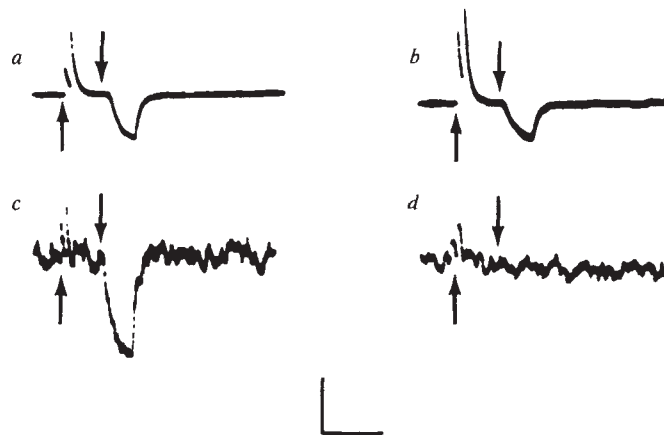


Fig. 1 Effect of fraction E on the lobster neuromuscular junction. In each trace, a pair of stimuli was applied to the excitatory axon (eliciting excitatory postsynaptic potentials) at the upward arrow; a train of stimuli (60 s^{-1}) was applied to the inhibitory axon (eliciting a summated inhibitory postsynaptic potential) at the downward arrow. *a*, Control; *b*, *c*, *d*, 17, 24, 28 min, respectively, after addition of $9\text{ }\mu\text{g}$ of fraction E. The excitatory potentials are potentiated in *b*, and the inhibitory potential is potentiated in *c*. In *d*, both the excitatory and inhibitory potentials are greatly reduced in amplitude. The ragged baseline in *c* and *d* is caused by a high frequency of spontaneous miniature potentials. Calibration: 2 mV, 1 s.

Venom glands were extracted from adult females of the black widow spider, *Latrodectus mactans tredecimguttatus*. Crude venom was prepared by homogenising two glands in 0.25 ml of lobster Ringer; venom was fractionated as previously described¹⁴. Intracellular recordings from the stretcher muscle of the lobster walking leg and from the frog cutaneous pectoris muscle were made using conventional techniques^{16,17}. Superficial fibres from the lobster muscle were exposed by chipping away the overlying shell and removing the connective tissue layers. A small pool ($\sim 0.2\text{ ml}$) was formed around the exposed fibres with Vaseline and toxins were added to the pool. Cutaneous pectoris muscles were pinned out in a chamber ($\sim 3\text{ ml}$) to which toxins were added.

In the lobster, crude BWSV initially causes a potentiation of excitatory and inhibitory postsynaptic potentials, both of which are subsequently abolished, and a great increase in the frequency of miniature excitatory postsynaptic potentials⁶. Figure 1 demonstrates that these physiological effects can be elicited by application of $9\text{ }\mu\text{g}$ of fraction E. In contrast, Fig. 2 shows that at the frog NMJ, even after 2 h of exposure to $12\text{ }\mu\text{g}$ of fraction E, no increase in m.e.p.p. frequency is observed. However, subsequent treatment with $1\text{ }\mu\text{g}$ of α -latrotoxin rapidly causes a large increase in m.e.p.p. frequency (Fig. 2*d*). To test whether the apparent inactivity of fraction E on the frog NMJ in the above experiments was due to the large volume of fluid in the chamber ($\sim 3\text{ ml}$), some additional experiments were carried out in which fraction E was applied to small ($\sim 0.2\text{ ml}$) Vaseline-enclosed pools surrounding endplate regions. Even in these conditions, no rapid increase in m.e.p.p. frequency was observed, although after prolonged exposure (1–2 h) to a large dose ($36\text{ }\mu\text{g}$) of this fraction, high frequency bursts of m.e.p.ps were seen. This low level of activity is most probably attributable to slight contamination of fraction E by α -latrotoxin, as a similar level of activity is observed after prolonged exposure to low doses of α -latrotoxin, and such contamination can be demonstrated by SDS-polyacrylamide gel electrophoresis.

Although the lobster NMJ is sensitive to fraction E, Fig. 3 demonstrates that it is insensitive to α -latrotoxin. Following the addition of $2\text{ }\mu\text{g}$ of α -latrotoxin, no potentiation of evoked responses and no increase in the frequency of spontaneous miniature potentials were observed. Subsequent addition of $50\text{ }\mu\text{l}$ of crude BWSV, however, caused its usual effect, verifying that the preparation was still capable of responding to the crude venom.

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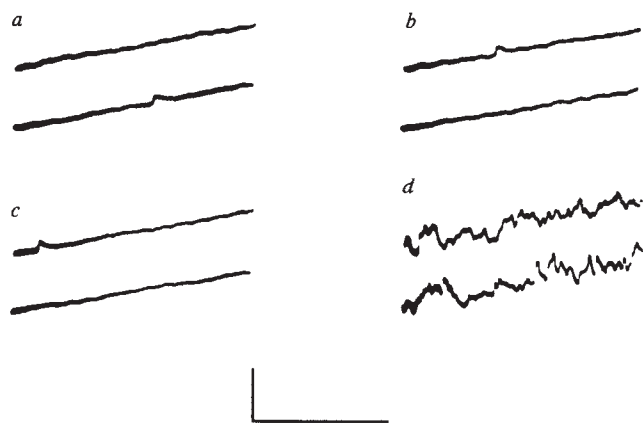


Fig. 2 The absence of an effect of fraction E on the frog neuromuscular junction. *a*, Control; *b* and *c*, 18 and 136 min, respectively, after addition of 12 µg fraction E to the bath. Individual m.e.p.s can be seen in *a*, *b* and *c*. α -Latrotoxin, 1 µg, was added 168 min after fraction E addition. *d*, 14 min after the α -latrotoxin application. A high m.e.p. frequency is evident in *d*. Calibration: 2.5 mV, 50 ms.

These experiments show that fraction E can account for all the physiological effects of BWSV on the lobster NMJ, whereas α -latrotoxin is inactive. In contrast, α -latrotoxin causes the release of every transmitter so far examined in vertebrates¹²⁻¹⁴. The present experiments also confirm that fraction E does not cause the release of transmitter at the frog NMJ¹⁴. Fraction E has previously been shown to be ineffective in stimulating the release of GABA from mouse brain slices¹³.

α -Latrotoxin binds with high affinity to some protein components in neuronal membranes¹⁸, possibly binds also to gangliosides¹⁹, and apparently acts, at least in part, by forming ionic channels^{20,21}. It seems possible, then, that the inactivity of α -latrotoxin at the lobster NMJ reflects an absence of toxin receptors on the lobster nerve terminal and/or an inability of the toxin to become incorporated into the lobster nerve terminal membrane. Similarly, membrane components that interact with fraction E may be present on nerve terminals of lobster but not of frog. We cannot rule out the possibility, however, that α -latrotoxin and fraction E do not have equal access to nerve endings of lobster and frog. The calcium ionophore X-537A, on

the other hand, seems to interact with both frog and lobster nerve terminals, as it is capable of increasing miniature potential frequency in both these preparations²².

BWSV causes transmitter release from insect NMJs^{9,10} and the component which affects the cockroach NMJ has a MW of 125,000 (ref. 10). It seems likely that this component is a constituent of fraction C described by Frontali *et al.*¹⁴, as this fraction is the only one to affect houseflies and cockroaches in their assay. Assuming this is true, the crude venom contains at least three separate protein components, each capable of causing transmitter release, but each demonstrating some degree of species specificity.

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Dopamine stimulates the degradation of gonadotropin releasing hormone by rat synaptosomes

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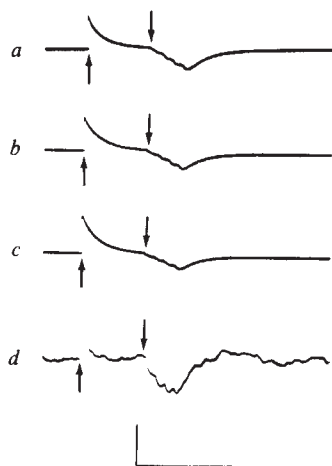


Fig. 3 The absence of an effect of α -latrotoxin on the lobster neuromuscular junction. In each trace, one stimulus was applied to the excitatory axon at the upward arrow; a train of four stimuli (10 s^{-1}) was applied to the inhibitory axon at the downward arrow. *a*, Control; *b*, and *c*, 10 and 20 min, respectively, after addition of 2 µg α -latrotoxin. 50 µl of crude BWSV was added 25 min after the α -latrotoxin. *d*, 10 min after the addition of the crude BWSV. The ragged baseline in *d* is due to a high frequency of miniature potentials induced by the venom. Calibration: 5 mV, 1 s.

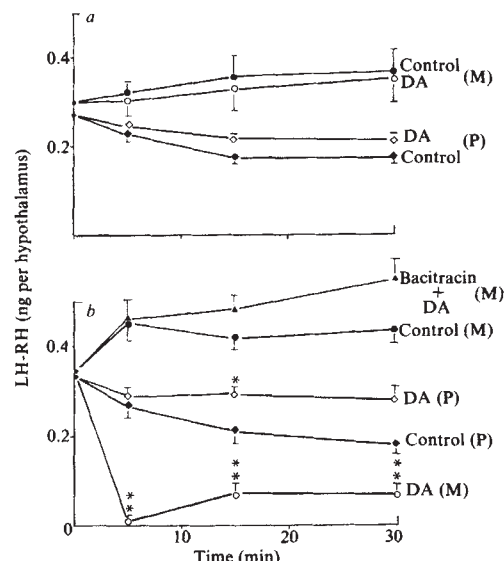
Immunohistochemical studies have shown the existence of synapses between nerve endings containing dopamine and gonadotropin releasing hormone (LH-RH) respectively in the hypothalamic median eminence¹. It is generally accepted that this transmitter is involved in the regulation of gonadotropin secretion as a modulator of LH-RH release, although the evidence is conflicting and both inhibitory and excitatory actions of dopamine have been reported^{2,3}. *In vitro* studies support the excitatory effect and low doses of dopamine have been shown to release LH-RH from incubated hypothalamic fragments^{4,5} and synaptosomes⁶. This report describes studies with nerve endings isolated from the rat hypothalamus in which dopamine at higher concentrations than those used previously has been found to stimulate the degradation of LH-RH but not thyrotropin releasing hormone (TRH). This mechanism seems to be physiological in that it is calcium-dependent, requires the structural integrity of the nerve endings and fluctuates with the reproductive state of the animal.

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Fig. 1 Effect of 10^{-4} M dopamine (DA) on the release of immunoreactive LH-RH from synaptosomes isolated from male (a) and female (b) rat hypothalamus. Adult male and female Wistar rats (200–300 g) were housed in a temperature-controlled animal room with lights on from 6.00 to 18.00 h. The animals were stunned and decapitated. The entire hypothalamus was rapidly removed and synaptosomes were isolated and suspended in Krebs-Ringer bicarbonate medium containing glucose, at a tissue concentration equivalent to 1 hypothalamus per ml, using methods described previously¹³. The synaptosomes were incubated at 37 °C in capped plastic vials for a preincubation period of 15 min before the addition of dopamine, added in 20 μ l of 1 mM ascorbic acid, to give a final concentration of 10^{-4} M. An equal volume of the ascorbic acid was added to control incubations. After incubation the synaptosomes were deposited by centrifugation (1,000g for 10 min) at room temperature and both the supernatant medium and synaptosomal pellets stored at -40 °C. Pellets were extracted with 0.5% Triton-X100 (ref. 14) and both LH-RH¹⁵ and TRH¹⁶ measured by immunoassay. Details of the specificity of the antibody used to measure LH-RH have been described elsewhere¹⁷. The antiserum does not recognise the peptide fragments produced after cleavage at the Gly⁶-Leu⁷ bond, the major mechanism for inactivation¹⁰, but it does cross-react with LH-RH after removal of the COOH-terminal glycine, the other major point of endogenous peptidase activity¹⁰. The antiserum against TRH does not cross-react with pyroGlu-His-Pro-OH, pyroGlu-His or pyroGlu-Met-ProNH₂ and in studies on endogenous TRH-like material separated by HPLC using several different solvent systems, cross-reaction occurs only against a single fraction with properties identical to synthetic TRH. Dopamine at the concentrations used in the experiments did not interfere with the immunoassays. This aspect was rigorously tested. Small differences in the standard curve for LH-RH were obtained in the presence of 0.5% Triton and two assays were run systematically, one including Triton which was used to estimate the LH-RH content of the extracted pellets. Curves show levels of immunoreactive LH-RH present in the incubation medium (M), and Triton extracts of the synaptosomal pellets (P). In preparations from both sexes approximately two thirds of the total tissue content of LH-RH was released after 30 min control incubation, a finding which we have discussed elsewhere¹⁸. In b the levels of LH-RH released in the presence of bacitracin alone were similar to those obtained with bacitracin plus dopamine, and have not been shown for the sake of clarity. Values are expressed as mean $\pm$ s.e.m. in 9 to 31 determinations. The statistical significance of differences was determined with a two-tailed *t*-test. Different from control groups at: * $P < 0.05$; ** $P < 0.001$.

Preliminary studies were carried out with hypothalamus from female rats at random stages of the oestrous cycle and from male animals. Figure 1 shows the basal release of immunoreactive LH-RH from synaptosomes, during a 30-min incubation period. Levels of LH-RH in the incubation medium were relatively high to begin with, because of release during the preincubation period, and increased only slightly during the next 30 min. The content of LH-RH in the synaptosomal pellet obtained after centrifugation showed a decline with time, indicating depletion of a releasable pool of the neuropeptide. In the presence of dopamine (10^{-4} M) there was a significant reduction in the level of LH-RH in the medium from female hypothalamic synaptosomes. This was maximal after 5 min, when almost all of the LH-RH released during the period of preincubation had disappeared. The disappearance of immunoreactive LH-RH from the medium after dopamine treatment is unlikely to be due to re-uptake or inhibition of release as the LH-RH content of the pellet was unchanged at this time, although the possibility of re-uptake coupled with intracellular degradation cannot be excluded. After 15 and 30 min of incubation the concentration of LH-RH in the synaptosomal pellets in the presence of dopamine was raised, but the preparation still showed a significant net loss of LH-RH ($P < 0.005$). Furthermore, the action of dopamine was blocked in the presence of bacitracin (5×10^{-4} M), an antibiotic peptide which competitively inhibits the degradation of LH-RH by peptidases⁷. In contrast to this effect of dopamine on LH-RH in synaptosomes isolated from female hypothalamus, dopamine did not reduce the LH-RH level in the medium from synaptosomes isolated from the male rat hypothalamus.

Subsequent experiments were carried out with synaptosomes from female rats at defined stages of the oestrous cycle and incubations with dopamine were carried out for 5-min periods. Figure 2 shows that although a significant effect of dopamine was obtained at every stage of the reproductive cycle, the degradation of LH-RH was most marked in the pro-oestrous preparation. Although the values are not shown, the concentrations of LH-RH in the synaptosomal pellets did not vary with different stages of the oestrous cycle and were not affected by the



addition of dopamine. Figure 2 also shows that in the same preparations there was a significant increase in the release of immunoreactive TRH in the presence of dopamine, an effect which we have also observed with nerve endings from sheep hypothalamus⁵. Dopamine did not alter the synaptosomal content of TRH.

The effect of dopamine on the degradation of exogenous LH-RH was also studied using synaptosomes isolated from

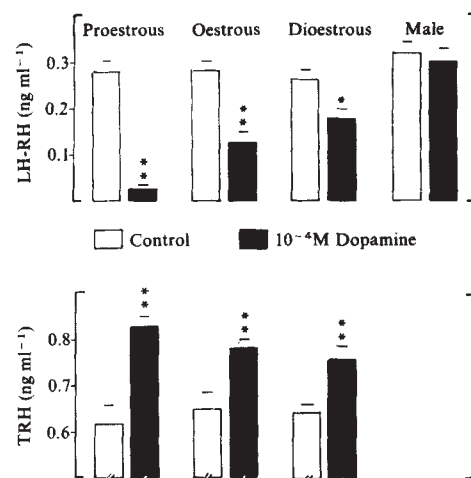


Fig. 2 Levels of immunoreactive LH-RH and TRH in the supernatant medium from synaptosomes isolated from female rat hypothalamus at different stages of the oestrous cycle. Daily vaginal smears were taken in the morning and only rats showing at least 2 or 3 successive regular 4-d cycles were used. Experimental conditions were identical to those described for Fig. 1. Values show levels of peptide present after 15 min preincubation followed by 5 min incubation in the presence of dopamine or ascorbic acid control vehicle, and represent the mean $\pm$ s.e.m. in 13 to 18 determinations. The statistical significance of differences was determined by the two-tailed *t*-test. Different from control groups at: * $P < 0.025$; ** $P < 0.001$.

female rats at random stages of the oestrous cycle. After pre-incubation, 10 ng of synthetic LH-RH was added, an amount almost 30-fold greater than endogenous levels released by the synaptosomes. Dopamine (10^{-4} M) stimulated the disappearance of exogenous LH-RH from the medium and bacitracin completely inhibited this effect (Fig. 3). Exogenous LH-RH was also added to suspensions of cerebral cortex synaptosomes incubated at a tissue concentration equivalent to 1/3 cortex per ml. Substantial degradation of LH-RH occurred in these conditions but dopamine did not have any significant effect on the rate of breakdown. The effect of removal of free calcium on dopamine-induced disposal of both endogenous and exogenous LH-RH by hypothalamic synaptosomes was also studied. When calcium was omitted from the Krebs-Ringer bicarbonate buffer, the degradation of LH-RH in the presence of dopamine was not observed (Fig. 3).

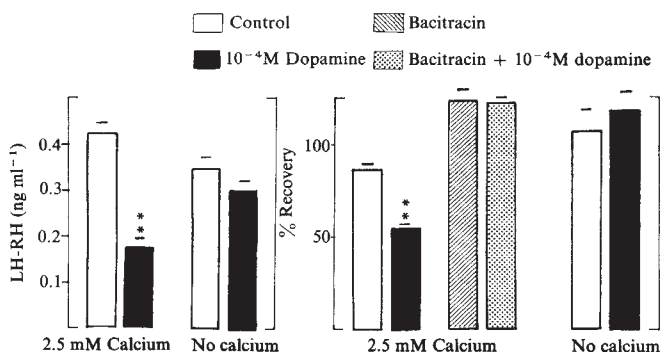


Fig. 3 Effect of calcium on the dopamine-induced degradation of endogenous (left) and exogenous (right) LH-RH. Percentage of recovery was calculated with each experiment from controls consisting of the various media incubated with 10 ng synthetic LH-RH. Values greater than 100% obtained when bacitracin was present or calcium was omitted, are presumably due to the decreased breakdown of endogenously released LH-RH in the medium. The synaptosomes were incubated for 30 min in the presence of LH-RH and other agents. The LH-RH content of the synaptosomal pellets was not significantly altered in the presence of dopamine. Values are expressed as mean $\pm$ s.e.m. in 9 to 32 determinations. The statistical significance of differences was determined by the two-tailed *t*-test. ** Different from control groups at $P < 0.001$.

The concentration of dopamine (10^{-4} M) required to stimulate the degradation of LH-RH by synaptosomes is relatively high. However, recently it has been reported that concentrations of dopamine in plasma from hypophysial portal blood are between 10^{-8} and 10^{-7} M (ref. 8). If this measurement is correct it is likely that much higher concentrations of dopamine occur in synaptic clefts or extracellular space surrounding dopaminergic nerve terminals. The concentration of acetylcholine in the neuro-muscular junction exceeds 10^{-4} M during stimulation⁹ and there is no reason to suppose that such levels of neurotransmitters are not found in synaptic clefts elsewhere.

When synaptosomes were ruptured by sonication, dopamine did not stimulate the degradation of 10 ng synthetic LH-RH added to the preparations, indicating that the mechanism of this effect does not involve a direct action on the enzyme. Also, the supernatant medium from incubated synaptosomes did not significantly degrade LH-RH. Together, these results suggest an extremely complex mechanism which requires the structural integrity of the nerve ending and in which dopamine, ovarian steroids and calcium are all involved. The existence of such a system for the degradation of LH-RH may partly explain the apparent lack of correlation between the distribution of LH-RH and the peptidases which degrade it. Previous studies¹⁰ have reported that less than 5% of LH-RH degrading activity is associated with the synaptosomal fraction, which contains most of the peptide. However, the membrane or cytosol preparations

used in such studies lack the cellular organisation that the dopamine-sensitive, synaptosomal mechanism requires.

That dopamine-induced breakdown of LH-RH observed in this study may reflect a physiological mechanism is suggested by: (1) the differential effect of dopamine in rats at different stages of the oestrous cycle; (2) the lack of effect of dopamine on LH-RH degradation on male hypothalamic synaptosomes; (3) the inability of dopamine to enhance LH-RH disposal in a calcium-free medium; (4) the absence of any action on TRH breakdown; and (5) the finding that dopamine did not stimulate the disposal of exogenous LH-RH by synaptosomes from cerebral cortex. We suggest, therefore, that in addition to controlling the release of hypothalamic LH-RH dopamine may acutely regulate the disposal of this hormone by controlling peptidase activity at the nerve terminal. Such a mechanism could be important with relation to both the endocrine functions of LH-RH in control of gonadotropin secretion and its reported actions on neuronal activity¹¹ and sexual behaviour¹². It could also have more general significance for the functional relationships between aminergic and peptidergic neurones in the central nervous system.

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Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells

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Morphological, physiological and pharmacological evidence indicates that opioid peptides, which in the brain are located intraneurally¹, may function as neurotransmitters. Similar evidence is not yet available for the opioid peptides that are stored in chromaffin cells of adrenal medulla²⁻⁵ and in axon terminals located in adrenal medulla and sympathetic ganglia³⁻⁵. The present report contributes evidence suggesting that the opioid peptides which are stored in the axon terminals of the splanchnic nerves located in adrenal medulla⁴ may function as

neuromodulators of the acetylcholine receptors located on chromaffin cells that are involved in catecholamine release. We support this functional role of the opioid peptides by showing that primary cultures of chromaffin cells of bovine medulla contain opiate receptors. When these receptors are occupied by specific agonists, the number of nicotinic receptors and the amount of catecholamine released by maximal doses of nicotine are reduced. Thus, like in other neuronal systems⁶⁻⁸ also in adrenal medulla, the action of opioid peptides is inhibitory. The specificity of this action is in part supported by the inability of opiate receptor agonists to reduce the Ca^{2+} -dependent release of catecholamines elicited by K^+ ions.

A highly purified primary culture of chromaffin cells was obtained from bovine adrenal medullae by a modification of the method described by Waymire *et al.*⁹. Approximately 10^8 chromaffin cells were obtained from a single bovine adrenal (for further details see Table 1 legend). These chromaffin cells can be kept in culture, without significant morphological or biochemical changes, for at least 2 weeks¹⁰. The chromaffin cells in culture retain the ability to release endogenous catecholamines in response to nicotinic receptor stimulation^{10,11}. The nicotine-induced release of catecholamines is Ca^{2+} dependent¹⁰.

Nicotinic receptor antagonists such as hexamethonium abolish this response^{10,11}, which is unaffected by pretreatment with blockers of muscarinic receptors¹⁰. This finding agrees with reports that in these primary cultures of chromaffin cells the

number of high-affinity binding sites for ligands (^3H -quinuclidinyl benzilate) of muscarinic receptor sites¹⁰ is very small.

The experiments reported here were carried out in cells maintained in culture for 1 week. To study catecholamine release, 10^6 cells were washed once with phosphate-buffered saline and suspended in 1 ml of Krebs-Ringer bicarbonate-glucose buffer (pH 7.2–7.4) and 0.5% bovine serum albumin at 37 °C. The content of catecholamines and their metabolites in the cells was measured by mass-fragmentography as previously reported^{12,13}, with minor modifications. The cells were homogenised in 100 μl of 1 M HCl solution containing 25 ng each of deuterated adrenaline, noradrenaline and dopamine. These internal deuterated amines were used to quantify the endogenous amines by direct comparison. Adrenaline (15 ± 0.8 nmol per 10^6 cells; $n=3$) represents approximately 75%, noradrenaline (3.4 ± 0.2 nmol per 10^6 cells; $n=3$) 17% and dopamine (1.3 ± 0.06 nmol per 10^6 cells) 7%, of the total catecholamine content. Only trace amounts of dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were detected. A rapid spontaneous release of catecholamines was observed during the first 5 min of incubation in Krebs buffer. During this period approximately 1–2% (0.2–0.4 nmol per 10^6 cells) of the total catecholamine stores was released. The spontaneous release of catecholamines decreased to 0.5% of the total cell content in the following 5-min period. During a 3-min exposure to nicotine (10^{-5} M) or a high potassium concentration

Table 1 Effect of morphine and other opiate receptor agonists on the release of endogenous catecholamines from cultured adrenal chromaffin cells

Secretory stimulus	Opiate receptor agonist	Catecholamine secretion (nmol per 10^6 cells)	%
Nicotine 10^{-5} M	None	2.0 ± 0.15	100
	Morphine	1.8 ± 0.15	89
	10^{-7} M	$1.5 \pm 0.06^*$	75
	10^{-6} M	$1.3 \pm 0.08^*$	65
	10^{-5} M	$1.1 \pm 0.09^*$	56
	10^{-4} M	$2.2 \pm 0.20^\dagger$	109
	Morphine	$1.5 \pm 0.10^\dagger$	75
	10^{-4} M + NTL 10^{-4} M	2.3 ± 0.20	100
Nicotine 10^{-5} M	None	1.9 ± 0.20	81
	β -Endorphin	$1.7 \pm 0.09^*$	76
	10^{-9} M	$1.6 \pm 0.10^*$	69
	10^{-8} M	$1.1 \pm 0.12^*$	49
	10^{-7} M	$2.2 \pm 0.19^\dagger$	94
	10^{-6} M	2.1 ± 0.15	100
	10^{-7} M + NTL 10^{-7} M	2.4 ± 0.29	116
Nicotine 10^{-5} M	None	$1.5 \pm 0.12^*$	70
	Met-enkephalin	$0.8 \pm 0.10^*$	38
	10^{-5} M	$0.7 \pm 0.07^*$	35
	10^{-4} M	$0.6 \pm 0.07^*$	28
	FK33-824	2.4 ± 0.23	109
	10^{-7} M	2.4 ± 0.30	108
KCl 56 mM	None	2.4 ± 0.23	109
	Morphine 10^{-4} M	2.4 ± 0.30	108

Adrenal chromaffin cells were isolated from fresh bovine adrenal glands as described by Waymire *et al.*⁹. Briefly, the procedure consists of: dissection of the adrenal medulla from the cortex, digestion of the interstitial tissue of adrenal medulla by retrograde perfusion with 0.25% collagenase, differential centrifugation and filtration, and differential plating to remove non-chromaffin cells. The chromaffin cells were cultured in F-12-10% fetal calf serum medium. The purity of the chromaffin cell preparation was established microscopically with the specific method for catecholamine fluorescence and Giemsa staining²³. The contaminant cells were always less than 5%. The chromaffin cells were able to maintain a constant level of catecholamines (20 nmol per 10^6 cells) and related enzymes for 1–2 weeks^{10,11}. Large numbers of cells could be purified with this procedure. Approximately 10^8 cells were obtained from a single bovine adrenal medulla. For the release studies approximately 10^6 cells per sample were used. The release of endogenous catecholamines from cultured chromaffin cells was measured by incubating the cell suspension at 37 °C for 2.5 min in Krebs-Ringer bicarbonate-glucose buffer (pH 7.2–7.4) and 0.5% bovine serum albumin with 2.2 mM CaCl_2 . The cell suspension was filtered through a Millipore filter after the incubation. The released catecholamines (adrenaline + noradrenaline) were extracted from the filtrate with 0.4 M perchloric acid and assayed fluorimetrically after Al_2O_3 adsorption¹⁴. 'Catecholamine secretion' represents the amount of catecholamines released (in 2.5 min) by nicotine or K^+ ions after subtracting the baseline release of catecholamines. The baseline release accounts for 20–30% of the total catecholamines present in the medium after nicotine or KCl stimulation. Each value is the mean $\pm$ s.e. of two or three separate experiments. In each experiment the effect of drugs (added 15 min before 10^{-5} M nicotine) was tested in groups of three separate samples. There was no significant release of the endogenous catecholamines by the opiate substances themselves at the doses used. Regression analysis of the dose-response curve for different opiate receptor agonists give the following coefficient of correlation (r^2) values: morphine, 0.99 ($P < 0.01$); β -endorphin, 0.88 ($P < 0.01$).

* Statistically significant ($P < 0.05$) when the treated groups are compared with the control group, using the *t*-test pair analysis.

† Statistically significant ($P < 0.05$) when morphine-treated groups are compared with the corresponding groups treated with morphine + naltrexone (NTL).

(56 mM), there was a large linear increase in the rate of catecholamine release which was 10 to 15 times greater than the spontaneous release rate.

During the 3-min stimulation period, 10–15% of the total catecholamine cell content was released. After this very high release there was a 3-min period of much slower release during continuous nicotine or potassium stimulation. We measured the relative proportion of adrenaline, noradrenaline and dopamine released before and after nicotine stimulation with or without opiate treatment by mass-fragmentography and found that the medium contained, in all the conditions studied, approximately 55% adrenaline, 45% noradrenaline and 1% dopamine. Only trace amounts of DOPAC and HVA were present. The catecholamine content remaining in the cells after the release experiments and the relative proportions of catecholamines present were mostly the same as those measured in control preparations. Due to the large amounts of catecholamines available in each sample (approximately 2–4 nmol in the supernatant and 20 nmol in the cells) and based on the mass-fragmentographic data indicating that practically only adrenaline and noradrenaline were released, we found it convenient in the experiments reported in Table 1 and Fig. 1 to assay the catecholamines (adrenaline + noradrenaline) with the spectrofluorimetric method¹⁴.

Table 1 shows that morphine reduces the release of catecholamines elicited by the addition of a maximally active dose (10^{-5} M) of nicotine. This inhibitory action of morphine is dose related and is abolished by the presence of naltrexone, a potent opiate receptor antagonist. The peptides β -endorphin and FK33-824 (a stable analogue of enkephalin¹⁵; Sandoz) also produced a statistically significant dose-related inhibition of the nicotine-stimulated catecholamine release (Table 1). These peptides are several orders of magnitude more potent than morphine or Met-enkephalin (Table 1). The action of opiate

receptor agonists cannot be due to an increase in the degradation rate of catecholamines because when ^3H -noradrenaline and ^3H -adrenaline were incubated for 3 min with the incubation media obtained from cells treated with opiate receptor agonists in the presence or absence of 10^{-5} M nicotine, no loss of ^3H -labelled catecholamines was measured. In addition, the effect of opiates cannot be due to changes in the cell catecholamine content because the cell content failed to change with the addition of opiate receptor agonists (20 ± 1.8 nmol per 10^6 cells in controls; 19 ± 1.5 nmol after 10^{-4} M morphine; 21 ± 2.0 after 10^{-7} M β -endorphin). These experiments suggest that opiate receptor agonists inhibit the release of catecholamines elicited by the stimulation of nicotinic receptors and that this action requires the availability of those receptor sites which are blocked by naltrexone. We then studied whether crude P_2 preparations of membranes obtained from chromaffin cell cultures contain specific sites for high-affinity binding of ^3H -Met-enkephalinamide and ^3H -naltrexone (Fig. 1). The K_D values calculated from Scatchard plots of the saturation curves are 0.9 nM for Met-enkephalinamide and 3.2 nM for naltrexone. These values are close to those calculated for opiate receptors present in brain membrane preparations¹⁶.

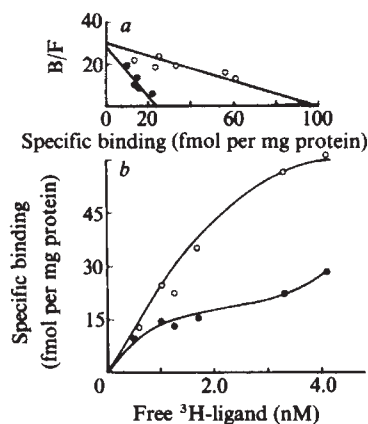


Fig. 1 Scatchard plots (a) and saturation curves (b) of ^3H -(D-Ala²-L-Met⁵)enkephalinamide (●) ($37.7 \text{ Ci mmol}^{-1}$), and ^3H -naltrexone (○) ($14.6 \text{ Ci mmol}^{-1}$, both NEN) binding to cell membranes. The cells were homogenised in 50 mM Tris-HCl (pH 7.0) and the homogenate was centrifuged at 12,000g for 15 min. The pellet was resuspended in 50 mM Tris-HCl (pH 7.0) and preincubated at 25°C for 1 h¹⁶. After the second centrifugation at 12,000g for 15 min, the pellet was resuspended in 50 mM Tris-HCl (pH 7.0). Specific binding was calculated by subtracting from the binding which occurred by incubating the pellet (approximately 100 μg protein) at 25°C for 1 h with ^3H -labelled ligand, the binding which occurred by incubating with ^3H -labelled ligand plus 10^{-3} M morphine in a total volume of 600 μl . After incubation the reaction was terminated by centrifugation at 12,000g for 15 min. The supernatant was discarded and the pellet was rapidly rinsed with 1 ml of ice-cold 50 mM Tris-HCl (pH 7.0). The pellet was dissolved in 50 μl of NCS (Amersham tissue solubiliser) before counting the radioactivity with a liquid-scintillation counter. Proteins were determined with the method of Lowry²⁴. $B/F = [\text{bound } ^3\text{H-ligand (fmol per mg protein)}]/[\text{free } ^3\text{H-ligand (nM)}]$.

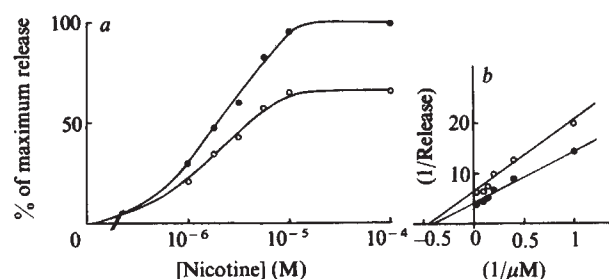


Fig. 2 Effect of morphine on the nicotine-induced release of endogenous catecholamines from cultured adrenal chromaffin cells. a, Release of endogenous catecholamines by various concentrations of nicotine in the absence (●) and presence (○) of 10^{-4} M morphine. b, Double-reciprocal plot of the data in a. The release of the endogenous catecholamines from cultured adrenal chromaffin cells was measured as described in Table 1. Morphine was added to the incubation medium 15 min before nicotine. Each point is the mean of two separate experiments in which triplicate samples were assayed. The error was less than 10% of the mean.

The release of catecholamines elicited by the stimulation of nicotinic receptors is inhibited by opiate receptor agonists (see Table 1) and by hexamethonium¹¹, but the mechanisms involved are different. Opiate receptor agonists act non-competitively (Fig. 2) whereas hexamethonium acts competitively^{11,17}. Figure 2a shows that the nicotine-induced catecholamine release is decreased by about 45% in cells preincubated with 10^{-4} M morphine (Fig. 2a). The double-reciprocal plot (Fig. 2b) of the dose-response curve indicates that the extent of the maximal response decreases, but that the concentration of nicotinic agonist which gives half-maximal response fails to change.

Morphine (10^{-4} M) does not inhibit the release of catecholamines induced by the depolarisation of the cells with KCl (Table 1). Thus, it seems that in the chromaffin cell membranes, the stimulation of opiate receptor changes the function of nicotinic receptors by a specific mechanism. To characterise this mechanism further, we have investigated whether the stimulation of opiate receptors located in the chromaffin cell membranes can influence the number of available nicotinic receptors. In these studies, ^{125}I - α -bungarotoxin was used as the ligand for nicotinic receptors^{18,19}. This ligand in a concentration of 10^{-8} M causes a 50% reduction of the release of catecholamine induced by 10^{-5} M nicotine. When morphine

(10^{-4} M) was added to the primary cultures of chromaffin cells it reduced the number of 125 I- α -bungarotoxin binding sites by approximately 80%. In untreated cells, the $B_{\max}$ for α -bungarotoxin binding was 3.1 fmol per mg protein; this value decreased to 0.65 in cells treated with morphine (10^{-4} M). The decrease in the $B_{\max}$ was not associated with a significant change in the affinity constant; the K_D values were 2.8 and 3.1 nM in normal and morphine-treated chromaffin cells, respectively. Similar results were obtained when the chromaffin cells were treated with FK33-824.

In conclusion, opiate receptors are present in the membrane of adrenal chromaffin cells. The activation of these receptors causes a non-competitive inhibition of catecholamine release elicited by the stimulation of nicotinic receptors. Our data indicate that β -endorphin, FK33-824 and Met-enkephalin reduce the action of nicotine by decreasing the number of available nicotinic receptors. This action is probably indirect and may involve an allosteric modulation of the recognition sites for ligands of nicotinic receptors. Based on these data, it is tempting to speculate that when the Met-enkephalin-like material present in terminals of splanchnic nerves³⁻⁵ is released by nerve impulses, it modulates the release of catecholamines induced by nicotinic receptor stimulation elicited by the concomitant release of acetylcholine. A recent report²⁰ indicates that substance P inhibits nicotinic activation of the chromaffin cells. However, the physiological significance of the latter experiment is difficult to establish because evidence was not provided for the presence of substance P or a substance P-like material in splanchnic nerve terminals. The presence of opiate receptors on chromaffin cell membranes may also suggest a role for opiate peptides released into the circulation from the pituitary²¹ or the adrenal cells^{1,22}. Although these cells respond to several opiate peptides, including β -endorphin, this sensitivity is very low relative to their actual concentration in blood.

The precise mechanisms whereby the endogenous ligands of opiate receptors modulate the number of functional nicotinic receptor sites present in chromaffin cell membranes is unclear. It seems, however, that in chromaffin cells the opiate peptides that probably co-exist with acetylcholine in splanchnic nerves can disable the function of nicotinic receptors. Hence, it may be interesting to consider the possibility that 'co-transmitters' function as 'enablers' of the receptor function for the primary transmitter.

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Amino acids evoke short-latency membrane conductance increase in pancreatic acinar cells

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Sodium-gradient-driven amino acid transport is regarded as an important mechanism for cellular uptake in general as well as in pancreatic acini¹⁻⁶. Electrophysiological experiments in kidney tubules and intestine have revealed that amino acids can cause sodium-dependent membrane depolarisation⁷⁻¹¹. The mechanism of this depolarisation is unknown¹⁰ as it is uncertain whether the depolarisation is accompanied by a membrane conductance increase^{9,11}. The pancreatic acinar tissue would seem to be an ideal system for investigating the electrophysiological mechanism of action of amino acids because (1) the pancreas is the most active amino acid-accumulating tissue¹², (2) the basic electrophysiology of the pancreatic acinar cells is well characterised¹³ and (3) a direct comparison can be made in the same cells between the electrical actions of the pancreatic secretagogues and the amino acids. We now show that L-alanine evokes a stereospecific membrane depolarisation accompanied by an increase in membrane conductance and that this membrane effect has a much shorter latency than the secretagogue response. The null (equilibrium) potential for the amino acid-evoked potential change corresponds to the sodium equilibrium potential.

Two separate KCl-filled microelectrodes were inserted into neighbouring cells in the same acinus of mouse pancreas (under visual control using a Zeiss (Jena) phase contrast microscope ($\times 400$) with long working distance) *in vitro* as previously described^{14,15}. Up to two extracellular micropipettes were used for local application of acetylcholine (ACh), L- or D-isomers of alanine, L-proline, L-valine or L-glutamate. The substances were ejected from the pipettes by ionophoresis using a similar procedure to that used for local application of peptide secretagogues¹⁶. The composition of the physiological saline solution, prewarmed to 37°C, flowing through the bath, was (mM): NaCl, 139; KCl, 4.7; CaCl₂, 2.56; MgCl₂, 1.13; D-glucose, 2.8; Tris, 3. pH was adjusted to 7.4 with HCl and the solution was gassed with 100% O₂.

Figure 1a shows membrane depolarisations evoked by microionophoretic application of L- and D-alanine. Although the same amino acid concentrations, stimulus parameters and relative positions of the micropipettes were used, the effect of D-alanine was small and inconspicuous compared with that of L-alanine. This was consistently found in 10 different experiments. The L-alanine-induced depolarisation resembled the depolarisation induced by secretagogues such as ACh, cholecystokinin-like peptides and bombesin-like peptides¹³⁻¹⁶. However, a close examination of the time course of the potential changes (Fig. 1b) revealed that the L-alanine-evoked membrane effect had a much shorter latency than the ACh-induced depolarisation. The minimum latency for muscarinic receptor activation of pancreatic acinar¹⁷ as well as other cells¹⁸ is about 0.3-0.5 s. Similar values have been found with peptide secretagogue-evoked effects¹⁶. In contrast, the latency for the action of L-alanine was <50 ms (the latency may well have been much shorter, but the artefact caused by the onset of the ejecting ionophoretic current unfortunately obscured the initial 50 ms of the potential recording). Also, in spite of the shorter delay of the amino acid effect (Fig. 1b) the rise-time was much longer than for ACh. These short-latency responses to L-alanine stimulation were observed in all 14 experiments of this kind carried out. The short-latency responses to L-alanine application show that there is no substantial diffusional barrier to close ionophoretic drug

application and that therefore the relatively long latency for the action of ACh is genuine. The long latencies for secretagogue actions in the pancreas may be related to the time taken for an intracellular messenger, calcium, to reach a sufficient concentration to initiate the response¹⁹. Although the effect of ACh was blocked by atropine (1 μ M), this had no effect on the L-alanine-evoked depolarisations (three experiments). Other amino acids were also tested, and L-valine (Fig. 2), L-proline, L-leucine and L-serine had effects similar to that of L-alanine. The latencies of the effects of L-valine and L-proline were determined in five experiments. In all these cases, short latencies of the same order of magnitude as found for L-alanine were observed. L-glutamate, however, had no effect on the bioelectrical properties in the five experiments in which it was tested.

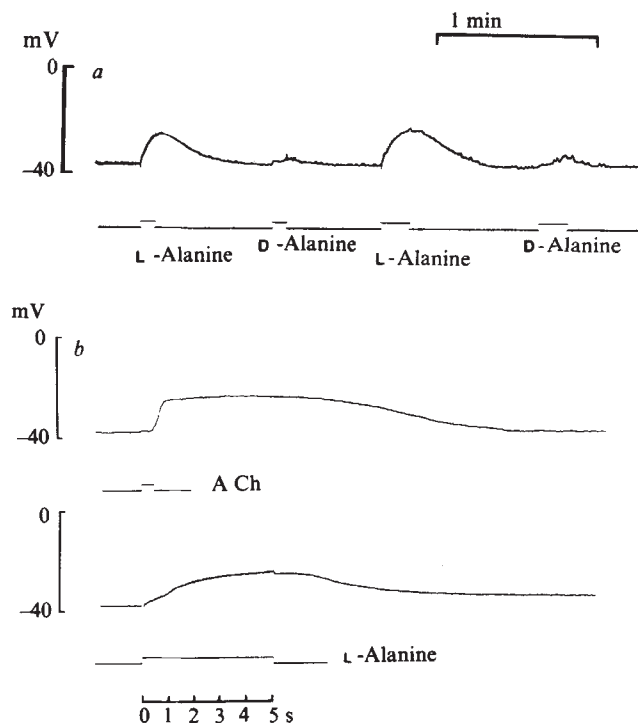


Fig. 1 *a*, Effects of ionophoretic L-alanine and D-alanine applications on the membrane potential in the same acinar cell (atropine (10^{-6} M) present). Micropipettes were filled with either 1 M L- or D-alanine (Sigma) dissolved in 0.9% NaCl (pH 4.0). The marker signals in the bottom trace indicate periods of L- or D-alanine ejection near to the surface of the impaled acinar cell, using positive ejecting currents of 100 nA and 5 s (short marker signals) or 10 s (longer marker signals) duration. No retaining current was used. *b*, Measurement of latency of responses to ionophoretic application of acetylcholine (ACh) and L-alanine from the same pancreatic acinar cell. Two extracellular micropipettes, filled with 2 M AChCl and 1 M L-alanine, respectively, were placed very close to the acinus impaled by the KCl-filled recording electrode. Upper trace: ACh stimulation was applied by a positive current of 80 nA (retaining current 20 nA) for 500 ms. Lower trace: L-alanine ionophoresis was applied by a positive current of 800 nA (no retaining current) for 5 s. The latency was taken as the interval between the start of the stimulus artefact and the take-off point arising from the depolarisation of the impaled acinar cell. A latency of 300 ms was found in the upper trace. The lower trace (L-alanine) shows the beginning of cell depolarisation less than 50 ms after the start of L-alanine ejection. The two traces are excerpts from a single continuous membrane potential recording from one cell.

We then tested the effect of superfusing the pancreatic acinar tissue with solutions containing known concentrations of L-alanine. At 0.1 mM, L-alanine caused a depolarisation of $3.3 \text{ mV} \pm 0.3$ (s.e.) ($n = 7$), a 1-mM concentration depolarised the acinar membrane by $7.3 \text{ mV} \pm 0.2$ ($n = 6$), and at 10 mM, L-alanine evoked a reduction in membrane potential of $17.6 \text{ mV} \pm 1.0$ ($n = 7$). The effect of 20 mM L-alanine was not significantly different from that obtained at 10 mM ($18.5 \text{ mV} \pm 0.5$ ($n = 5$)).

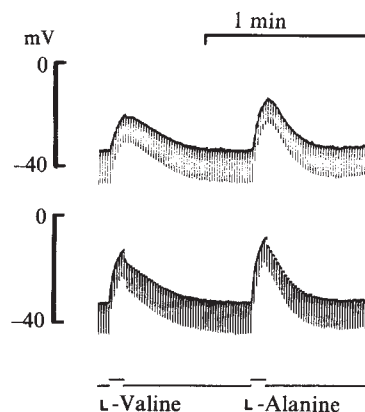


Fig. 2 Effects of microionophoretic application of L-valine and L-alanine on membrane potential and resistance in two electrically coupled neighbouring acinar cells. Current pulses (1 nA, 100 ms duration) were injected through the microelectrode recording the membrane potential represented by the lower of the two potential traces. Current pulses (500 nA and 5 s duration) were passed through the two extracellular micropipettes filled with 0.5 M L-valine or 1 M L-alanine, respectively, at marker signals in the bottom trace. The sudden small potential change corresponding to the end of ejecting current pulses seen in the lower potential trace represents the current artefact. (It is not seen in the upper trace, because that membrane potential was recorded using a differential amplifier.) Resting input resistance was 12 M Ω in this case. During the ionophoretic applications of L-valine and L-alanine the resistance was reduced to 10 and 8.5 M Ω , respectively.

The depolarisation evoked by L-alanine or L-valine was accompanied by a reduction in membrane resistance (Fig. 2). This was a consistent finding in the several hundred experiments carried out with L-alanine and the eight experiments with L-valine. Dose-response curves for L-alanine-evoked depolarisation and resistance reduction followed each other closely. The maximal reductions in input resistance obtained were to about 50% of the pre-stimulation value.

Replacing all the superfusion fluid Na by Tris (16 experiments) or Li (6 experiments) in all cases resulted in an immediate reduction in the amplitude of the L-alanine-evoked depolarisation. This effect was immediately reversible on re-admission of Na to the bath.

Figure 3 shows an attempt to determine the null (reversal) potential for the L-alanine-evoked potential change. In all seven experiments of the kind shown in Fig. 3, an approximately linear relationship between membrane potential and amplitude of L-alanine-evoked depolarisation was observed. An actual reversal of the potential change could not, however, be obtained because it proved impossible to attain membrane potentials

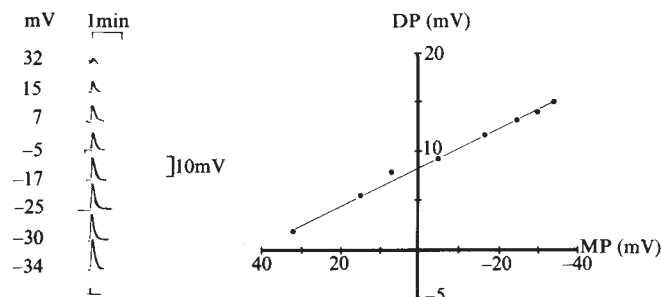


Fig. 3 To the left is shown the effect of L-alanine ionophoresis (1,000 nA ejecting 2 s duration) on the membrane potential of an acinar cell at different levels of resting potential (figures written to the left of individual records) in the same cell. Higher and lower resting potentials were obtained by passing direct hyper- or depolarising current, respectively, through a microelectrode inserted into an electrically coupled neighbouring cell. The L-alanine-evoked potential changes, plotted as a function of resting potential, are shown to the right. DP, depolarisation; MP, membrane potential.

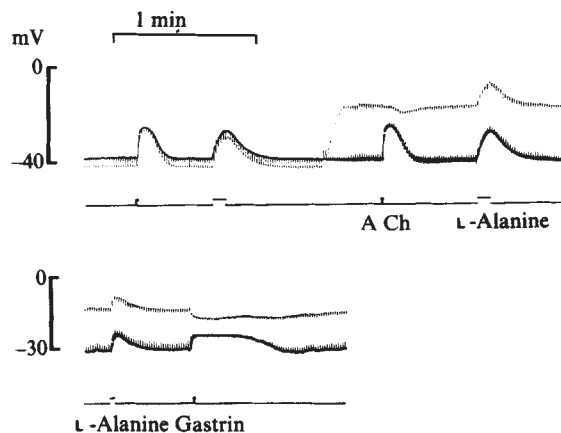


Fig. 4 Comparison of the membrane effects of L-alanine with ACh or gastrin in the same cells. In the upper trace ACh and L-alanine applied from two separate micropipettes by ionophoresis (80 nA, 0.5 s and 800 nA, 5 s, respectively) evoked depolarisations of similar size. During the first ACh and L-alanine stimulation, small hyperpolarising current pulses were repetitively injected through a microelectrode inserted into a neighbouring cell. Thereafter, the polarity of the injected current pulses was switched and the intensity increased (to 7 nA, 100 ms). In this way, the action of ACh and L-alanine could be simultaneously assessed at the spontaneous resting potential (heavy trace) (about -40 mV) and the artificially lowered potential (interrupted trace) (about -14 mV). The reduction in resting potential from -40 to -14 mV just reversed the polarity of the ACh-evoked membrane change, whereas the L-alanine-evoked depolarisation was only slightly reduced. The lower trace shows a similar comparison between L-alanine and gastrin actions in another cell. Gastrin was added directly (3.5 nmol) to the bath. At -30 mV, both substances evoked depolarisation. At -13 mV, the gastrin response was reversed but the L-alanine response was only slightly reduced in amplitude.

beyond +40 mV by direct current injection. Extrapolation of the linear curve in Fig. 3 to the intersection with the membrane potential axis (x-axis) revealed a null (reversal) potential of +40 mV. Values ranging from +20 to +60 mV were found in the other six experiments. These values are vastly different from the reversal potential of -10 to -20 mV repeatedly observed for the potential changes evoked by several different pancreatic secretagogues^{14,16}. The null potential for the secretagogue action of about -15 mV has previously been shown to be due to

increases in both chloride and sodium conductance, with some contribution also from potassium¹⁴. Figure 4 shows that current-evoked membrane depolarisations just sufficient to cause reversal of the effects of ACh or gastrin fail, in the same cells, to reverse the L-alanine-evoked depolarisation. In mouse pancreatic segments superfused *in vitro*, the intracellular sodium concentration, $[Na]_i$, is about 42 mM (ref. 20), therefore, the Na equilibrium potential (E_{Na}) at 37 °C is about +33 mV. The null (reversal) potential for the action of L-alanine seems to be very close to this value. Null potentials (extrapolated) of +20 to +40 mV were estimated for L-valine and L-proline in four experiments similar in nature to that shown in Fig. 3.

Several L-amino acids are able to evoke marked membrane depolarisation of pancreatic acinar cells. This phenomenon is very different from the previously demonstrated electrophysiological effects of pancreatic secretagogues¹³⁻¹⁶ in that the latency is much shorter and the null (reversal) potential much more positive (+40, rather than -15 mV). The clear resistance reduction (Fig. 2), together with the finding that the null potential has a value close to the calculated Na equilibrium potential, indicates that L-alanine, and several other amino acids, open up conductance pathways in the plasma membrane mainly permeable to Na.

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Induction of interferon in AKR mice by murine leukaemia viruses

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A high percentage of AKR mice develop spontaneous leukaemia which has been shown to be associated with the early expression of ecotropic murine leukaemia virus (MuLV)¹ and the subsequent expression of xenotropic² as well as polytropic³ MuLVs. Generally, mice infected with any one of several groups of viruses, including MuLVs, have been shown to produce interferon (IF)⁴. However, we report here that AKR mice produce no IF, despite that fact that infectious, endogenous MuLV is expressed in these mice from birth⁵.

Three possible reasons for the lack of IF in AKR mice can be considered: (1) a genetic deficiency in their ability to produce IF in response to MuLV; (2) the particular MuLV produced by AKR mice is not an inducer of IF; (3) the chronic production of

MuLV in AKR mice interferes in some way with the induction or production of IF. The last possibility is suggested by previous studies demonstrating that mice chronically infected with lymphocytic choriomeningitis virus produce substantially less IF in response to inoculation of Newcastle disease virus than do uninfected controls⁶.

These three possibilities were examined in the experiments described in Table 1 legend. AKR, B10.K, BALB/c and NFS mice were infected with virus derived from preleukaemic AKR mice (AKR-P) or with other MuLVs. Sera were collected from these mice 6 and 24 h later and IF titres in the sera were measured by a modification⁷ of the microplate method described by Havell and Vilček⁸. Mice from strains other than AKR were included in this study because, unlike AKR, they do not chronically produce endogenous, infectious ecotropic MuLV. In addition, NFS ($Fv-l^a/Fv-l^a$) mice were included to eliminate any possible effect of $Fv-l$ (ref. 9), as N-tropic AKR-P virus does not replicate well in cells from B10.K or BALB/c mice because of their $Fv-l^b/Fv-l^b$ genotypes. The Gross virus (GV) and Friend virus (FV) used in these studies were N→NB tropic, and thus replicate equally well in mice from strains homozygous for $Fv-l^a$ or $Fv-l^b$.

The results shown in Table 1 indicate that there is no genetic deficiency in AKR mice to prevent production of IF and that chronic expression of AKR-P does not interfere with IF production in AKR mice, as these mice produce IF after inoculation with GV or FV. It seems, however, that AKR-P virus does

Table 1 Induction of interferon in different mouse strains by various murine leukaemia viruses

Virus	Time (h)	Titre of interferon in:			
		AKR	B10.K	BALB/c	NFS
—	0	<15	<15	<15	<15
GV	6	<15	<15	<15	<15
AKR-P	6	<15	<15	<15	<15
FV	6	20	30	20	<15
BALB/c ext.	6	<15	<15	<15	<15
GV	24	720	180	120	360
AKR-P	24	<15	<15	<15	<15
FV	24	420	600	240	260
BALB/c ext.	24	<15	20	<15	<15

AKR/J mice (Jackson Laboratory), aged 2–3 months, and C57BL/10-H-2^k (B10.K), BALB/c or NFS (bred in our colony) were inoculated intraperitoneally with 0.2 ml of various virus preparations. Virus preparations were 10% cell-free extracts of spleen from: leukaemic BALB/c mice infected with N→NB tropic Gross virus (GV) or N→NB tropic Friend virus (FV); preleukaemic AKR mice (AKR-P); or normal BALB/c. Virus titres (plaque-forming units per ml) determined in the XC assay⁹ were: FV, 2.2×10^6 ; GV, 4×10^4 ; AKR-P, 6×10^4 ; BALB/c, 2×10^0 . Mice were bled at 6 and 24 h after inoculation of virus. Sera of two or three mice per group were pooled and assayed for the presence of IF by the microplate method of Havell and Vilček⁷, as modified by Armstrong⁸. Briefly, 50,000 L cells were added to wells containing serial twofold dilutions of sera. The cells were challenged 24 h later with encephalomyocarditis virus at a multiplicity of 0.2. Changes in cytopathology were recorded 24 h later. Titres were expressed as the highest dilution which protected half of the cells against virus-induced cytopathology. This was expressed as 1-ml volumes and corrected against mouse IF reference standard G002-904-511 distributed by the Reference Reagents Board of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. The values given are the average of results from two sets of assays. Neutralisation of IF activity of the samples was as described by Paucker *et al.*¹³. The IF induced by GV and FV in all strains of mice was totally neutralised by sheep anti-type 1 mouse fibroblast IF antiserum.

not induce IF, because none of the strains of mice studied produced detectable amounts of IF after inoculation with this virus. As the titre of AKR-P virus was similar to that of the GV preparation as measured in the XC assay¹⁰ (see Table 1 legend), the lack of IF induction by AKR-P virus cannot have been caused by differences in the amount of virus inoculated.

One possibly relevant distinction between the non-IF-inducing AKR-P virus and the IF-inducing GV is that the latter was derived from leukaemic AKR mice. It was therefore necessary to determine whether a recent virus isolate from leukaemic AKR mice (AKR-L) was capable of inducing the production of IF. The results shown in Table 2 indicate that the AKR-L virus preparation, similar to the AKR-P preparation, does not induce IF production in AKR (*Fv-lⁿ/Fv-lⁿ*) or DBA/2 (*Fv-lⁿ/Fv-lⁿ*) mice. The difference in abilities of GV and FV on the one hand, and of the AKR-P and AKR-L viruses on the other, to induce IF, may reflect a change in the GV and FV during laboratory passage. It is interesting that, although live AKR viruses do not

Table 2 Induction of interferon by virus from leukaemic and preleukaemic AKR mice

Virus	Time (h)	Titre of interferon in	
		AKR	DBA/2
—	0	<15	<15
AKR-P	24	<15	<15
AKR-L*	24	<15	<15
GV	24	80	135
FV	24	150	90

For techniques, see Table 1 legend. Titres are the average of the results from two sets of assays.

* 10% Cell-free extract of spleen from leukaemic AKR mouse (AKR-L). Virus titre as determined in the XC assay: 3×10^4 ml⁻¹.

induce IF, a previous study has demonstrated that exogenous IF is effective in inhibiting endogenous MuLV production in AKR mice¹¹.

It is thought that during gestation of AKR mice a small number of cells begin to express virus spontaneously¹². Virus produced by these cells then infects other cells which in turn produce virus, and a cascade effect results. If AKR virus did induce IF production by infected cells, the progression of the infection might be aborted because of the protective effect of IF. Thus, it is interesting to speculate that in selecting for a strain of mice with a high incidence of spontaneous leukaemia, Furth may have selected for a strain (AK) which produces an MuLV that, although sensitive to IF, does not induce IF.

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Induction of secondary cytotoxic T lymphocytes by liposomes containing HLA-DR antigens

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It is evident that the gene products of the major histocompatibility complex (MHC) have a crucial role in the induction of cytotoxic T lymphocytes (CTLs) and as target antigens for CTLs^{1–6}. Assessment of the precise molecular requirements for CTL induction and recognition, however, is difficult when intact cells are used as targets. Recently, in several model systems, it has been demonstrated that, after detergent solubilisation, cell-surface antigens can be isolated that retain the ability to stimulate a secondary CTL response. CTL stimulating activity has been isolated by detergent solubilisation from murine tumour cell plasma membranes^{7,8} which co-purified with H-2 antigens through lentil lectin affinity chromatography and gel filtration^{9,10}. Highly purified HLA-A and HLA-B antigens, from the human lymphoblastoid cell line JY, have been isolated and incorporated into well characterised liposomes¹¹. Spleen cells from mice primed to JY cells developed cytolytic activity, specific for JY targets, after co-culture with these HLA-liposomes¹². In these experiments it was observed that membranes which had been solubilised with deoxycholate and then dialysed to remove the detergent (reconstituted membranes) always stimulated greater cytolytic activity than did HLA-liposomes. At the time it was suggested that this disparity might reflect the loss of other antigens involved in CTL stimulation as a consequence of HLA-A and HLA-B purification. Data are presented here which demonstrate that other antigens are indeed able to induce secondary CTLs, independently of the HLA-A and HLA-B antigens. The results suggest that this activity is present on HLA-DR-associated molecules.

C57BL/6 mice were primed intraperitoneally with 2×10^7 viable JY cells 1–2 months and again 1 week before *in vitro* culture¹². Spleen cells were then isolated and co-cultured for 5 days with various liposome preparations. Cytolytic activity was assessed by incubation of the effector cells with ^{51}Cr -labelled target cells. Reconstituted membranes were prepared by solubilisation of JY membranes in 0.5% deoxycholate, centrifugation at 100,000g for 1 h, and dialysis of the supernatant¹¹. The 100,000g supernatant contained 80% of the membrane protein and 90% of the membrane phospholipid. Reconstituted membranes were selectively depleted of certain proteins by passage of the detergent-solubilised supernatant through anti- β_2 -microglobulin-Sepharose or lentil lectin-Sepharose columns before dialysis. Material specifically eluted from the columns was reconstituted by dialysis after addition of JY lipid. Liposomes containing purified HLA-A and HLA-B antigens were prepared as previously described¹¹. Briefly, the dried lipid was dispersed together with the purified protein in 0.5% deoxycholate, and the detergent was then removed by dialysis. Data indicate that the material eluted from the lentil lectin column also becomes associated with phospholipid vesicles (K. Parker, V.H.E. and J.K., unpublished). Equivalent amounts of dialysed material (based on A_{280} and phospholipid content) were centrifuged at 100,000g for 1 h and resuspended in a volume corrected for the amount of dilution which occurred on the columns. The pellets generally contained 80–90% of the detergent-solubilised phospholipid and protein¹³.

The CTL-stimulating activity of these various preparations was assessed on JY targets at an effector: target ratio of 100:1 (Table 1). Reconstituted membranes stimulated far greater cytolytic activity than did liposomes containing only purified HLA-A and HLA-B antigens (compare samples 1 and 5). In addition, the flowthrough from the anti- β_2 column (sample 3) demonstrated CTL-stimulating activity. As assessed by inhibition of complement-dependent cytotoxicity, this preparation contained less than 0.005% of the original amount of the

Table 1 Only a portion of CTL-stimulating activity is removed by anti- β_2 -microglobulin

Sample	HLA ($\mu\text{g ml}^{-1}$)	% Lysis of JY
1. Reconstituted membranes	20	52
2. One pass over anti- β_2 column	0.5 (0.2%)	20
3. Two passes over anti- β_2 column	0.001 (0.005%)	16
4. One pass over lentil lectin column	0.9 (4.4%)	0
5. HLA-A and HLA-B liposomes	20	26

Fractions were prepared by solubilisation of 2.5 mg of JY membranes in 2 ml 10 mM Tris, 0.14 M NaCl and 0.5% deoxycholate, pH 8.0, followed by centrifugation at 100,000g for 1 h. The supernatant was either dialysed immediately against 10 mM Tris, 0.14 M NaCl and 5 mM CaCl_2 , pH 7.8, or passed through anti- β_2 -microglobulin-Sepharose or lentil lectin-Sepharose columns (0.5 ml each). The anti- β_2 -microglobulin column had been equilibrated in solubilisation buffer, and the lentil lectin column was equilibrated in 10 mM Tris, 0.14 M NaCl, 0.1% deoxycholate, 75 μM CaCl_2 and 75 μM MnCl_2 , pH 8.0. The flowthrough fractions were monitored by A_{280} , and fractions representing at least 85% of the recovered A_{280} were pooled. The extent of dilution of this material compared with the starting material was assessed by phospholipid analysis according to Ames²⁶. The pooled material was then dialysed as described above. After dialysis all fractions were centrifuged at 100,000g for 1 h and resuspended in 1 ml 10 mM Tris, 0.14 M NaCl and 5 mM CaCl_2 , pH 7.8, per ml of original detergent-solubilised supernatant. Aliquots of 50 μl was added to each 2-ml culture well. Lysis is expressed as per cent specific release of ^{51}Cr (ref. 2).

HLA-A and HLA-B antigens. However, passage of solubilised membranes over a lentil lectin column was sufficient to remove stimulating activity entirely.

Table 2 presents evidence that solubilised membrane proteins which flow through the anti- β_2 column and specifically bind to lentil lectin can stimulate CTL activity when incorporated into liposomes. SDS-polyacrylamide gel electrophoresis of fractions from Table 2 shows that although the flowthrough material from the anti- β_2 -microglobulin and lentil lectin columns contained most of the protein bands present in the reconstituted membranes, the lentil lectin eluate consisted primarily of two polypeptide chains of molecular weights 29,000 and 34,000, with no detectable HLA-A and HLA-B band (Fig. 1). These polypeptides have previously been shown by various immunological and protein chemical criteria to correspond to the HLA-DR alloantigens, which are the human analogues of the murine Ia antigens^{14,15}. Although other minor protein bands are present in the lentil lectin eluate, the presence of HLA-DR antigens as the major component of this material, coupled with previous reports of Ia- or HLA-DR-specific allogeneic CTL, suggest that these antigens represent the non-HLA-A and -B CTL-stimulating activity seen here.

We are currently investigating the specificity of the CTLs induced by these HLA-DR-containing liposomes. It is possible that CTLs are induced with specificity to HLA-A and HLA-B antigens or that these liposomes induce CTLs with specificity for HLA-DR antigens. These possibilities were tested as follows:

Table 2 The CTL-stimulating material which flows through the anti- β_2 -microglobulin column can be specifically bound to and eluted from lentil lectin

Sample	% Lysis of JY
1. Reconstituted membranes	54
2. Two passes over anti- β_2 column	38
3. Above passed through lentil lectin column	4
4. 2% α -methyl mannoside eluate of lectin column	31
5. HLA-A and HLA-B liposomes	24

The procedure was the same as that described in Table 1 legend, except that the detergent-solubilised material was passed through the lentil lectin column only after passage through the anti- β_2 -microglobulin column. In addition, the lentil lectin eluate was reconstituted after addition of 1 mg ml^{-1} JY lipid.

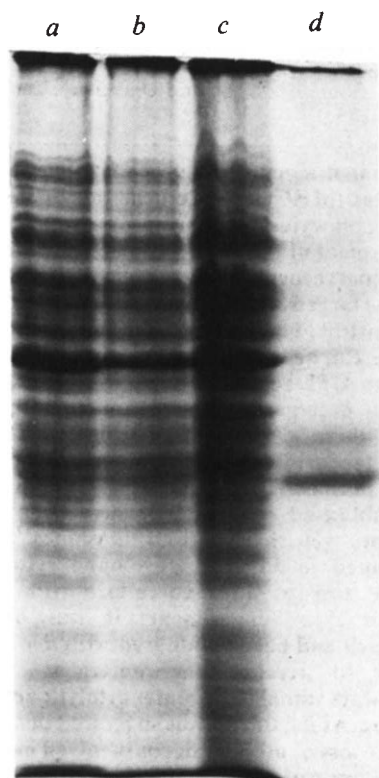


Fig. 1 12% SDS-polyacrylamide gel electrophoresis patterns of fractions from Table 2. Lane a, detergent-solubilised membrane supernatant; lane b, anti- β_2 -microglobulin flowthrough; lane c, lentil lectin flowthrough; lane d, lentil lectin eluate.

CTLs were induced with liposomes containing either HLA-A and HLA-B or HLA-DR. These CTLs were assayed on Daudi target cells, which are HLA-A and HLA-B negative but HLA-DR positive¹⁶. Only CTLs induced with HLA-DR liposomes lysed Daudi, suggesting that these CTLs have specificity for HLA-DR antigens (Table 3). We confirmed that the cytolytic activity observed on Daudi targets resided in the T-cell population by two criteria: cytolytic activity was eliminated by treatment of cells with anti-Thy 1.2 serum plus complement, and the effluent cells from nylon wool column passage (which removed $\geq 95\%$ Ig⁺ cells) retained cytolytic activity (data not shown). Preliminary experiments indicate that an antiserum raised against the highly purified HLA-DR complex (anti-p 29,34)¹⁷ and which recognises only these two chains by immunoprecipitation¹⁴ blocks target cell lysis by CTLs stimulated with HLA-DR-containing liposomes.

Table 3 CTL induced with HLA-DR containing liposomes lyse Daudi target cells

Stimulator	Target	
	JY	Daudi
HLA-A and HLA-B liposomes	66	5
HLA-DR liposomes	78	55

Spleen cells from C57BL/6 mice primed with JY were stimulated with either HLA-A and HLA-B or HLA-DR liposomes. These CTL were then assayed after 5 days on either JY or Daudi targets.

There have been several reports that CTLs can be generated to mouse allogeneic Ia antigens¹⁸⁻²⁰. It has also been observed that these Ia antigens are recognised by CTLs independently of H-2K and H-2D antigens^{21,22}. Recently, several studies have shown that CTLs were generated in human mixed lymphocyte cultures between donors differing at the HLA-D/DR but not HLA-A and HLA-B loci²³⁻²⁵. These CTLs only lysed targets bearing HLA-DR region antigens (monocytes and Epstein-Barr virus-transformed peripheral blood lymphocytes).

In conclusion, non-HLA-A and -B cell surface antigens have been isolated from JY cells that are able to stimulate primed xenogeneic mouse spleen cells to exhibit cytolytic activity. These antigens seem to be HLA-DR antigens, the human equivalent of the mouse Ia antigens, and preliminary data suggest that the CTLs induced are specific for HLA-DR antigens. Further studies are in progress to define the specificity of the CTLs induced and to determine whether other biological functions are associated with these isolated HLA-DR antigens.

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The joining of V and J gene segments creates antibody diversity

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The variable regions of mouse kappa (κ) chains are coded for by multiple variable (V) gene segments and multiple joining (J) gene segments. The V κ gene segments code for residues 1 to 95; the J κ gene segments code for residues 96 to 108 (refs 1-3). This gene organisation is similar to that encoding the V λ regions⁴. Diversity in V κ regions arises from several sources: (1) there are multiple germ-line V κ gene segments and J κ gene segments; (2) combinatorial joining of V κ gene segments with different germ-line J κ gene segments; and possibly, (3) somatic point mutation, as postulated for V λ gene segments⁵. Also, from a comparison of the number of germ-line J κ gene segments and amino acid sequences, it has been suggested that J κ region sequences may be determined by the way V κ and J κ gene segments are joined^{2,3}. This report supports this model by directly associating various J κ sequences with given V κ gene segments.

The number of germ-line J κ gene segments has been estimated in two ways. A region of DNA containing J κ gene segments has been characterised by DNA sequence analysis and was found to include five J κ (or J κ -like) gene segments, only four of which have been found to be expressed as J segments^{2,3}. Additional J κ gene segments have not been found, although their presence cannot be excluded rigorously. Alternatively, the number of J κ gene segments may be determined by counting the number of distinctive RNA precursor patterns associated with different κ chain mRNAs⁶. Since the κ -mRNA precursors contain the intervening sequence between the J κ and C κ segments, they have a characteristic size depending on which J segment is used, the largest precursor corresponding to the J κ segment most distal to the C κ gene and the smallest to the most proximal J κ segment. On this basis the RNA precursor patterns for 20 different myeloma tumours were classified into four different categories, corresponding to the four different J κ gene segments that these chains use. The distances between the C κ gene and the various J κ gene segments deduced from the analysis of precursor patterns in NZB plasmacytomas agree with the distances determined by restriction mapping and DNA sequence analysis of BALB/c germ-line DNA. A previously suspected discrepancy⁶ has been resolved by a more accurate calibration of the mobility data.

We have analysed κ chains of the V κ 21 group from the NZB mouse strain. To date ten different J κ amino acid sequences have been observed in twenty V κ 21 examples (Fig. 1). Thus the

Fig. 1 J segments associated with $V_{\kappa}21$ chains from NZB myeloma antibodies. J segments are numbered according to their processing category⁶. This is based on the relative sizes of the κ -mRNA precursors, as determined by gel-electrophoretic analysis of poly(A)⁺ nuclear RNA. These precursors are approximately 5.3, 5.0, 4.4 and 4.1 kilobases for categories I, II, III and IV, respectively. The amino-terminal sequences (40 residues) of PC8701, PC8982 and PC10916 were determined on a modified Beckman sequenator as described previously¹¹ and were found to be representatives of the $V_{\kappa}21$ subgroups, D, C and C, respectively. The J segment sequence of these κ chains were determined as follows: each κ chain was subjected to mild acid cleavage¹² of the aspartylproline between residues 94 and 95. The peptide mixture was sequenced without separation after the amino-terminal peptide was blocked by addition of fluorescamine¹³. The rest of the sequences are taken from ref. 1. The germ-line J segments are defined as described in the text. J_1 , J_2 , J_3 and J_4 are coded for by the BALB/c embryo J gene segments termed J_1 , J_2 , J_4 and J_5 in ref. 2 or those termed J_5 , J_4 , J_2 and J_1 in ref. 3. The PC2413 J segment may be coded for by a germ-line J gene (or variant of a J gene) unique to the NZB mouse.

	Sequences of J segments associated with $V_{\kappa}21$ segments		Frequency	Processing category
	Germline			
		96		
		107		
J_1		W T F G G G T K L E I K R	5/20	I
J_2		Y —————	3/20	II
J_3		F ————— S —————	2/20	III
J_4		L ————— A ————— L —————	4/20	IV
$J_?$		W ————— D ————— E —————	1/20	I
Variant				
J_1^* PC6684		R —————	1/20	I
J_1^* PC7940		P —————	1/20	I
J_2^* PC7132		(P) Y —————	1/20	II
J_3^* PC8701		[] ————— S —————	1/20	III
J_4^* PC8982		L ————— A ————— E ————— L —————	1/20	IV

number of J_{κ} sequences appears to be greater than the number of germ-line J_{κ} gene segments. There are three types of J_{κ} sequence. First, four distinct types (J_1 , J_2 , J_3 and J_4) correspond to those coded for by four of the germ-line J_{κ} gene segments in BALB/c DNA. Each of these J_{κ} sequences has been observed two or more times in the NZB $V_{\kappa}21$ regions (Fig. 1). These J_{κ} sequences also have been observed in BALB/c κ chains⁷ indicating that NZB and BALB/c germ-line J_{κ} gene segments code for the same J_{κ} regions. A second type of J_{κ} sequence differs by a single residue from those of the germ-line J_{κ} gene segments; these are termed variant J_{κ} sequences. There are five J_{κ} sequences of this type and each has been observed only once.

There are two possible explanations for the variant J_{κ} sequences: (1) they arise from somatic diversification which occurs at the junctional region during the joining of V_{κ} and J_{κ} gene segments or (2) they are encoded by additional germ-line J_{κ} gene segments that are absent from or have not yet been detected in the BALB/c genome. The former model predicts that the precursors of the mRNAs coding for these variant J_{κ} segments would belong to a category typical of one of the four germ-line examples; the latter model predicts a unique mRNA precursor for each variant. A third type of J sequence which occurs in the exceptional PC2413, differs from the other germ-line J_{κ} gene segments by at least two nucleotide substitutions.

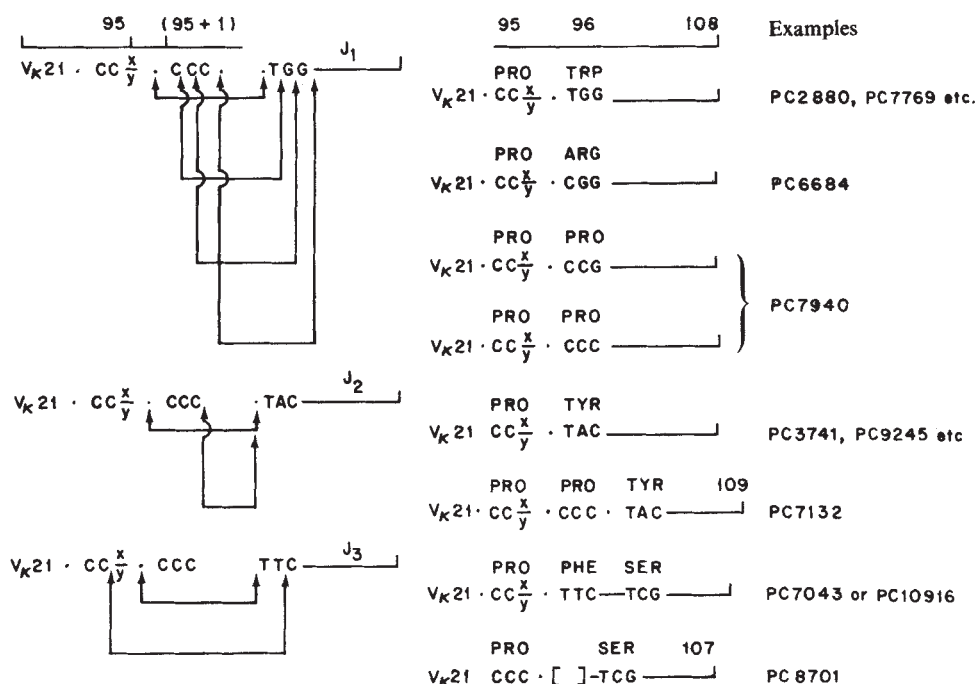


Fig. 2 Fusion of $V_{\kappa}21$ genes and J gene segments. The fusion can occur to join V and J gene segments exactly to form a contiguous V-J gene. Such genes will have germ-line J region sequences as in the case of PC2880, PC3741 or PC7043 and other examples¹. Fusion to J genes might include nucleotides in the intervening sequence (95 + 1). If this codon is CCC for all $V_{\kappa}21$, then the variant sequences, PC6684, PC7940 and PC7132 can be explained by fusion to a J gene (PC7132), within a J gene (PC6684 and PC7940) or within a V gene (PC8701).

Differences of this extent raise the issue of whether the PC2413 J_{κ} sequence might be encoded by a fifth germ-line J_{κ} gene segment in the NZB mouse.

An analysis of the RNA processing patterns for the tumours producing κ chains with variant J_{κ} sequences indicates that each pattern belongs to one of the four categories characteristic of germ-line J_{κ} gene segments (Fig. 1). Thus it appears that these variant J sequences arise during the process of V_{κ} - J_{κ} joining.

Knowing the size of the mRNA precursor for a J_{κ} variant allows us to infer the kind of somatic mutation that could have produced the variant sequence. For example, both PC6684 and PC7940 mRNAs exhibit the J_1 type precursor. Their J segments differ from the germ-line J_1 segment at position 96, presumably due to modifications of the tryptophan codon 96. PC7132 arose from the insertion of a proline codon before the J_2 gene segment. PC8701 resulted from a deletion of the phenylalanine codon at the beginning of J_3 gene segment. In addition to this junctional diversity, random point mutations either in the germ line or during somatic differentiation may occasionally occur (for example, PC8982).

The κ -mRNA precursor in PC2413 appears to belong to the category characteristic of the germ-line J_1 segment. Thus, if the J sequence of this tumour is actually encoded by a unique NZB germ-line J_{κ} segment, this segment would have to be located very close to the J_1 segment, or alternatively, be associated with a second, as yet unidentified, C_{κ} gene.

The nature of most J_{κ} variants suggests that they may be due to variations arising from the V-J joining process because the substitutions, mutations, insertions or deletions generally occur at the beginning of J_{κ} segments. These variations may be explained by two base substitutions (for example, tryptophan to proline) as well as the insertion or deletion of codons. Thus this variation does not appear to arise merely by single-base somatic mutation. Leder and Tonegawa and their coworkers have recently observed identical inverted repeat sequences at the 3' end of the V gene segment and at the 5' end of the J gene segment. They have hypothesised that these inverted repeats may form paired stem structures that juxtapose the V and J gene segments and that recombination may occur across the base of this stem at slightly different points to generate V-J junctional diversity (refs 2 and 3 and Fig. 2). This site-specific recombinational model appears to explain the V-J junctional diversity that we have noted in the $V_{\kappa}21$ chains.

By whatever mechanism V gene segments are translocated to J gene segments, a continuous DNA sequence coding for the entire V region must be generated⁴. Since all mouse κ chains studied to date have proline at residue 95 (CC^y), a V_{κ} gene segment translocation to the J_1 gene segment (tryptophan (TGG) at residue 96) will result in the contiguous DNA sequence, CC^y TGG at the V-J joining site (Fig. 2). Such a translocation will create a V_{κ} gene segment that is associated with the J_1 germ-line gene segment. This type of translocation explains five out of the twenty $V_{\kappa}21$ regions observed (Fig. 1). Likewise, similar translocations between $V_{\kappa}21$ gene segments and the J_2 , J_3 and J_4 gene segments can explain the nine additional examples of germ-line J segments (Fig. 1).

The variant J segments may arise by recombination within rather than between the V gene segment codons for positions 95 or 95 + 1 and their counterparts in the J gene segments (Fig. 2). Codon 95 + 1 is CCC in several V_{κ} gene segments^{2,8}. If the (95 + 1) codon is CCC for all $V_{\kappa}21$ gene segments, the type of variation that may occur at the V-J joining site is limited. Amino acid substitutions at residue 96 for a $V_{\kappa}21$ - J_1 translocation can only be arginine (CGG) or proline (CCG) (Fig. 2). An amino acid insertion would result in an extra proline codon before the J gene segment. In addition, deletions of residue 96 could occur. As shown in Fig. 2, the variants observed so far fit the predictions of this model. If there is variation at the (95 + 1) codon, a greater variety of substitutions could occur at residue 96³. Obviously similar variation could occur in the (96 - 1) codon of the J gene segment. Additional analyses of $V_{\kappa}21$ chains are

under way to define more completely the amino acid sequence variability at the V-J junctional site.

In our survey of $V_{\kappa}21$ chains, the frequency of κ chains expressing variant J segments relative to those expressing germ-line J segments is high (5 out of 20). This frequency could be high because the N-terminus of the J_{κ} segment is important for combining-site size and shape⁹ and antigen selection of specific antibodies amplifies mutations that occur at a low frequency. (The uniformity of J_{λ} segments¹⁰ may mean that variation in this region does not affect the complementarity of antibodies with λ chains.) Alternatively, this frequency may be a true reflection of the rate of variation by this mechanism.

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Translation of globin messenger RNA by the mouse ovum

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It has been demonstrated that the *Xenopus* oocyte can translate rabbit haemoglobin messenger RNA (mRNA) following microinjection of the message into the cell¹. The *Xenopus* oocyte has since been shown to be capable of translating a variety of messenger RNAs from different species²⁻⁴. This system has proved useful in understanding the mechanism of message translation and has also provided information about the translation capability of the *Xenopus* oocyte^{5,6}. Several other cell types, including HeLa cells and fibroblasts, can also translate exogenous message injected into the cell^{7,8}. However, there have been no reports of injection of mRNA into oocytes or fertilised one-cell ova of mammalian species. Nevertheless, the latter system could be of considerable use in studying the processing of exogenous messages in a mammalian system undergoing development, as well as providing insight into the way the early embryo processes injected messages and the protein products of such messages. We report here the results of injecting message into the fertilised one-cell mouse ovum and show that both mouse and rabbit globin mRNA are translated in this system.

Fertilised one-cell mouse ova were collected following superovulation from hybrid C57 × SJL females on the day of the vaginal plug by techniques described previously⁹. The ova were collected in Brinster's medium for ovum culture (BMOC-2, see ref. 9) and the cumulus cells removed with hyaluronidase (300

units ml^{-1}) in phosphate-buffered saline⁹. After washing to remove cumulus cell debris, the ova were maintained in BMOC-2. Purified mRNA for these experiments was prepared by the method of Gorski *et al.*¹⁰ except that polysomes were dissociated with SDS and applied directly to oligo(dT)-cellulose as described by Krystosek *et al.*¹¹. For injection, the ova were placed on a depression slide and held with a blunt pipette as shown in Fig. 1. The tip of the injector pipette was filled with a solution of mRNA in water (1 mg ml^{-1}) and a small amount of solution was injected into each ovum. The average volume injected was determined by injecting ^3H -inulin into several groups of ova and measuring the radioactivity found in each ovum. On the basis of these measurements, the average volume injected into each ovum was estimated to be 13 pl. Some of the ova underwent lysis during the 15–30 min after removal of the injector pipette and release of the ovum from the holder. The survival rate was 30–40%, and it was found that injection of the ova in medium containing cytochalasin B ($5\text{--}10 \mu\text{g ml}^{-1}$) approximately doubled the chance of survival. Each group of 20–40 injected ova was placed in BMOC-2 containing a mixture of five ^3H -labelled amino acids (leucine, lysine, phenylalanine, proline, tyrosine; specific activities $75\text{--}120 \text{ Ci mmol}^{-1}$; Amersham Searle) for 15–17 h, during which time approximately one third of the one-cell ova divided into two cells. Generally, about 80% of one-cell ova will cleave during this period, but the high level of radioactivity necessary for labelling decreased the number of ova undergoing cleavage. After labelling, the ova were washed three times in media containing non-radioactive amino acids, placed in a $6 \times 60 \text{ mm}$ tube and stored at -70°C . To determine whether globin had been synthesised from the injected message, non-radioactive globin marker was added to the tube, and the proteins were separated by two-dimensional high-resolution electrophoresis¹².

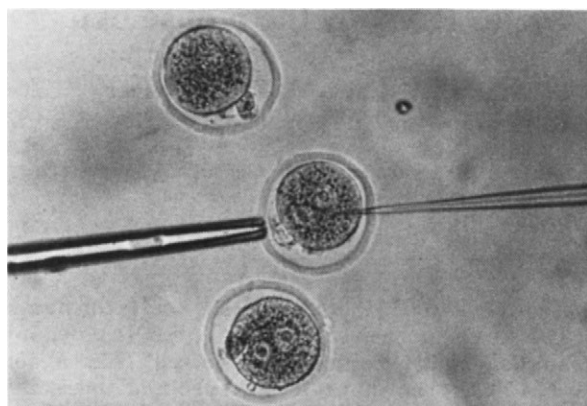


Fig. 1 Injection of one-cell mouse ovum with mRNA. The ovum was held with a blunt suction pipette ($5 \mu\text{m}$ i.d., $20 \mu\text{m}$ o.d.). The o.d. of the injector pipette was $2\text{--}3 \mu\text{m}$, and the tip was sharpened either by chipping or grinding. The ova were maintained on a depression slide in culture medium under silicone oil. The injection solution drop, from which the injector pipette was filled, was adjacent to the medium containing the ova. The technique was similar to that used for injecting cells into blastocysts¹⁵.

Figure 2 shows a typical fluorograph of the electrophoretic separation of synthesised proteins following injection of ova with mouse globin mRNA. Two major radioactive spots, indicated by arrows, co-migrated with non-radioactive globin marker prepared from mouse blood. Injection of message into mouse oocytes yields a similar pattern (unpublished). The other spots on the fluorograph are endogenous proteins. On control gels from ova not injected with mRNA there are no radioactive spots in the area of globin migration, indicating the absence of

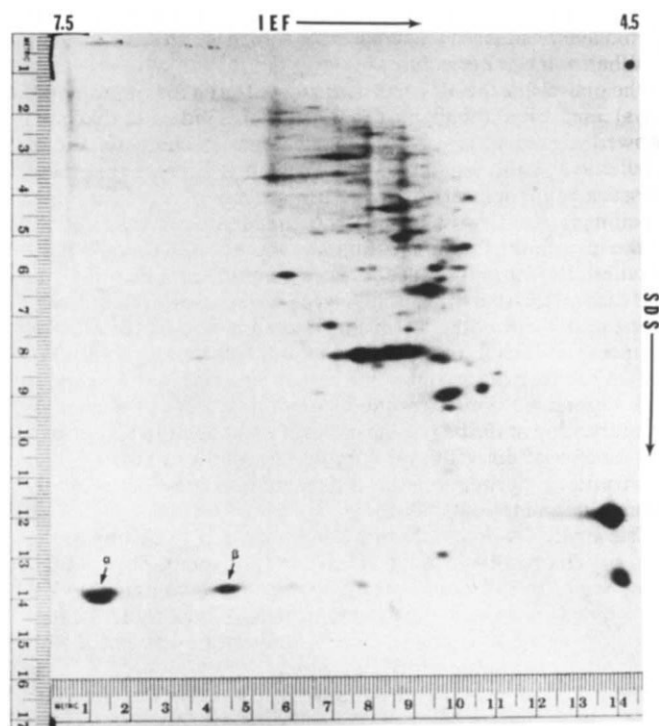


Fig. 2 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with mouse globin mRNA and incubated in ^3H -labelled amino acids. Isoelectric focusing (IEF) was from left (pH 7.5) to right (pH 4.5) and SDS-polyacrylamide electrophoresis was from top to bottom. The technique used was basically that of O'Farrell, as previously described^{12,16}. The IEF was carried out with pH 3–10 ampholines. Marker proteins on the right margin are lactoglobulin (top, MW = 18,400) and methaemoglobin (bottom, MW = 16,000). The two radioactive spots indicated by the arrows coincide with the position of the non-radioactive globin marker protein prepared from mouse blood. α and β indicate the positions of α - and β -chains of globin.

endogenous labelled protein in this area that might confuse the interpretation of the fluorographs (Fig. 3). When the non-radioactive markers are separated on isoelectric focusing (IEF) gels and stained for protein, each of the major spots is composed of two closely migrating but clearly distinct bands. This separation, although less apparent in the second dimension, can sometimes be seen in the radioactive spots such as the most basic of those in Fig. 2. No globin marker bands are present in the IEF gel at a more basic position than those shown in Fig. 2. The absence of such bands was confirmed by non-equilibrium pH gradient electrophoresis (unpublished observations); in this procedure the migration is from the acidic end and can be stopped at various positions before reaching the basic end¹³. Thus, the technique used for the egg proteins is capable of identifying the location of all the globin chains synthesised from the injected message.

When rabbit mRNA is injected into the ova, the pattern is slightly different. As shown in Fig. 4, only one major globin spot is present. This major spot seems to be composed of two sub-bands, similar to the situation for the mouse globin. However, because of the high level of radioactivity, it generally appears as one spot in the photographs of the fluorograph. By reference to purified globin markers for mouse α - and β -chain and for rabbit α - and β -chain, the large basic spot in Figs 2 and 4 was identified as α -globin for both the mouse and rabbit mRNA. The more acidic spot from the mouse globin mRNA is β -globin. No β -globin spot, which would be located in approximately the same position as the mouse β -globin, can be detected in Fig. 4.

However, other studies suggest that a low level of rabbit β -globin synthesis does occur (unpublished observations).

When ova were injected with either mouse or rabbit message at the one-cell stage, cultivated *in vitro* to the blastocyst stage (5 days) and then labelled with ^3H -amino acids, fluorographs showed no indication of globin synthesis. In fact, preliminary studies suggest that globin synthesis is not detectable 48 h after message injection. However, some globin is synthesised when labelling begins 24 h after message injection. A precise half life of the injected message can only be determined following more detailed investigation to identify the saturating level of message and the lag time before the message is active. However, it is clear that the mRNA half life in mouse ova is considerably shorter than that in *Xenopus* oocytes, where globin message has been shown to retain its activity for 2 weeks or longer¹⁴. Clearly, the cell type containing the message profoundly influences its stability. These studies do not indicate whether the message is degraded or is converted to a non-translatable form. Although the former possibility seems the more likely, the presence of masked message in the embryo makes the latter an intriguing possibility.

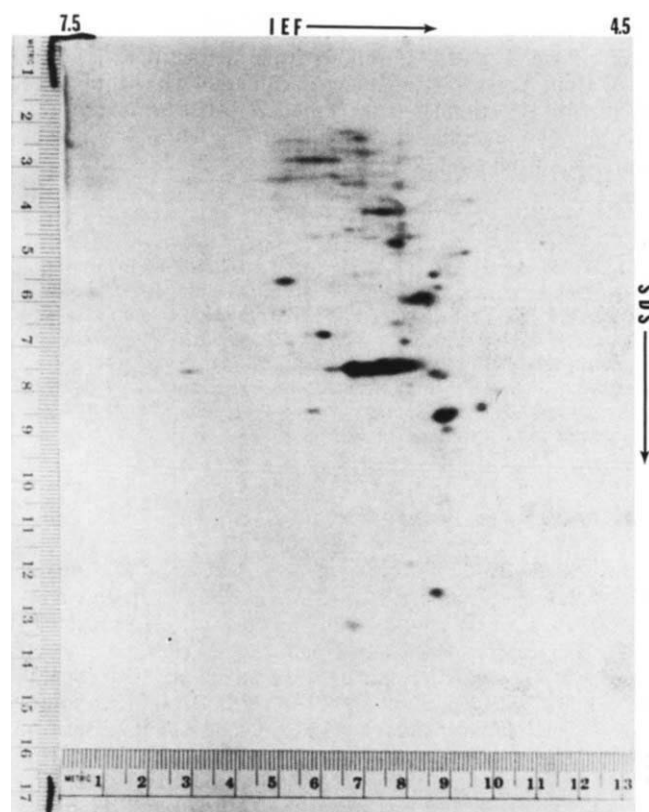


Fig. 3 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with water. Techniques used were similar to those for Fig. 2. No radioactivity appears in the area of globin migration.

These observations on the translation of globin message by the mouse ovum identify an important new system in which to study the activity of mRNA and the synthetic products of the message. Although having some similarities to the *Xenopus* oocyte, the system shows several differences. For instance, the mouse cells are mammalian rather than amphibian and are actively dividing rather than dormant or static as is the *Xenopus* oocyte. The unique characteristics of each system may provide different answers to the same question. The large difference

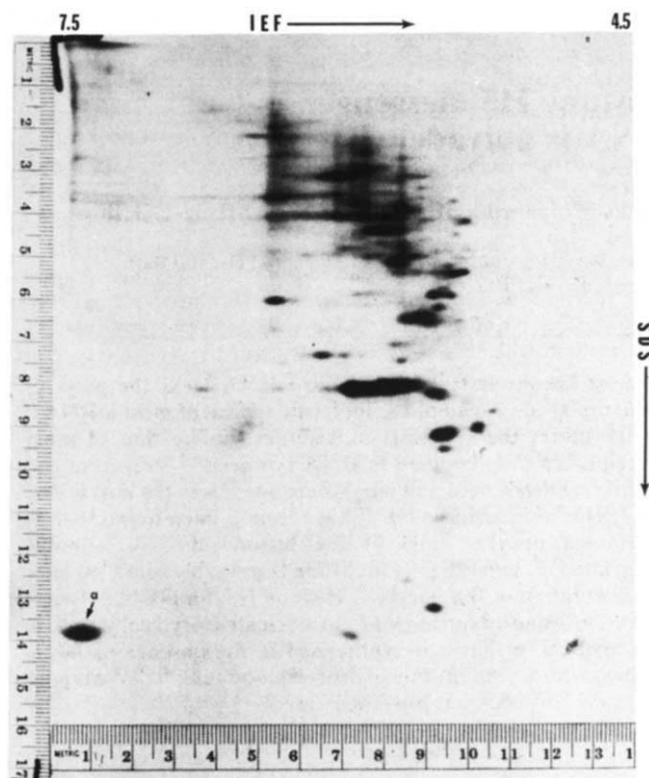


Fig. 4 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with rabbit globin mRNA. Techniques used were similar to those for Fig. 2. The single radioactive spot indicated by the arrow coincides with the position of the non-radioactive globin marker protein prepared from rabbit blood. α Indicates the position of the α -chain of globin. No β -chain was detected on the fluorograph.

found between the two cell types in the stability of injected globin message is one example. Another is the preponderance of synthesis of α - relative to β -chains in the mouse ovum; the relative synthesis is just the opposite in the *Xenopus* oocyte. The injection of exogenous messages into the mouse ovum should help in understanding message stability and activity in the developing mammalian embryo, as well as providing a means of studying translational activity and specific protein stability in the embryo.

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Histone H5 messenger RNA is polyadenylated

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In most known systems^{1,2}, histone mRNA lacks the poly(A) sequence at the 3' end of the molecule typical of most mRNAs. Furthermore, the synthesis of histones, unlike that of most proteins, is tightly coupled to DNA synthesis³⁻⁵. Nevertheless, histone synthesis occurs in amphibian oocytes in the absence of DNA synthesis⁶. Moreover, it has recently been found that in amphibian oocytes most of the histone mRNA is polyadenylated⁷⁻⁹, and the polyadenylate is probably removed during maturation of the oocyte¹⁰. Histone H5, an H1-like tissue-specific histone occurring only in nucleated erythrocytes¹¹, is also atypical in that it is synthesised in the absence of DNA synthesis during maturation of the red blood cells^{12,13}. We report here that H5 mRNA is polyadenylated.

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Fig. 1 H5 mRNA binding to poly(U)-Sephacrose and oligo(dT)-cellulose. *a*, 7.5 µg of immature chicken erythrocyte polysomal RNA, phenol extracted²¹ from polysomes enriched for H5 message by immunoprecipitation¹⁶ was fractionated by poly(U)-Sephacrose chromatography²². The unbound material, the 5% and 90% formamide eluates were, after ethanol precipitation, dissolved in 10 µl water. Aliquots (2 µl) of each of these fractions were analysed by translation in a rabbit reticulocyte cell-free translation system (NEN) using ³⁵S-L-methionine as label. Aliquots of the lysate were analysed on 15%-19% SDS polyacrylamide gels²³ and exposed by fluorography²⁴ on pre-sensitised film²⁵. Lane 1, total enriched polysomal RNA translated in the cell free system; lane 2, H5, labelled *in vitro*, by reductive methylation²⁶; lane 3, no RNA added to the lysate; lane 4, polysomal RNA not bound in 0.2 M NaCl in ETS buffer (0.01 M Tris-HCl pH 7.5, 0.01 M EDTA, 0.2% SDS); lane 5, polysomal RNA eluted by 5% formamide in ETS; lane 6, RNA eluted by 90% formamide in ETS. *b*, immature chicken erythrocyte polysomal RNA was phenol extracted as above from polysomes¹⁴ and immunoprecipitated as described by Groner *et al.*¹⁵. The RNA was purified by size fractionation on a sucrose gradient before being chromatographed on oligo(dT)-cellulose (T 3 Collaborative Research) and oligo(dA)-cellulose (T 6 Collaborative Research). The RNA was analysed by translation as above. The 0.4 M NaCl and 0.1 M NaCl solutions referred to below included 0.01 M Tris-HCl pH 7.4, 0.001 M EDTA, 0.5%, SDS. Lane 1, *in vitro* labelled H5; lane 2, RNA before oligo(dT)-cellulose chromatography; lane 3, RNA unbound in 0.4 M NaCl; lanes 4 and 7, RNA eluted with 0.1 M NaCl; lanes 5 and 8, RNA eluted with sterile water; lane 6, RNA not bound to oligo(dA)-cellulose in 0.4 M NaCl. *c*, Part of the RNA used in *b* was translated as described and the PCA-soluble translation products were precipitated by rabbit antibodies directed against H5 (manuscript in preparation). The labelled proteins were analysed as in *a*. Lane 1, an aliquot of the total translation assay; lane 2, the translation products precipitated by anti-H5.

RNA from anaemic chicken blood was enriched in H5 mRNA by immunoprecipitation of isolated polysomes¹⁴, essentially as described by Groner *et al.*¹⁵. In one experiment polysomes were isolated and immunoprecipitated as described by Scott & Wells¹⁶. The result of passing total polysomal RNA, enriched for H5 mRNA, over poly(U)-Sephacrose is shown in Fig. 1*a*. In this sample there is little evidence of translatable H5 mRNA in the unbound material (lane 4) and only a trace in the 5% formamide eluate (lane 5). The bulk of the translation product, which migrates at the same mobility as *in vitro*-labelled H5, occurs in the translation product of the fraction eluted by 90% formamide (lane 6). In Fig 1*b* a similar result is shown with a different sample of RNA which had been further purified by size fractionation on a sucrose gradient, and subjected to oligo(dT) cellulose chromatography. Although a small fraction of the H5 mRNA activity does not bind to oligo(dT) cellulose (lane 3) most of the translatable activity appears in the bound fraction eluted with distilled water (lane 5). To ensure that the binding of H5 mRNA was not due to an interaction with the cellulose support we passed the RNA over oligo(dA) cellulose (Fig. 1*b* lanes 6-8). The H5 mRNA showed no affinity for this support (compare lanes 6 and 8).

In all these experiments the H5 mRNA behaved in much the same way as the contaminating poly(A)-containing globin mRNA. That the translation products migrating with the mobility of H5 were indeed H5 follows from the fact that: (1) only RNA from polysomes immunoprecipitated with antibodies specific for H5 stimulated the rabbit reticulocyte to produce protein migrating with the mobility of H5, which was absent when RNA from total polysomes was used; (2) the cell-free H5

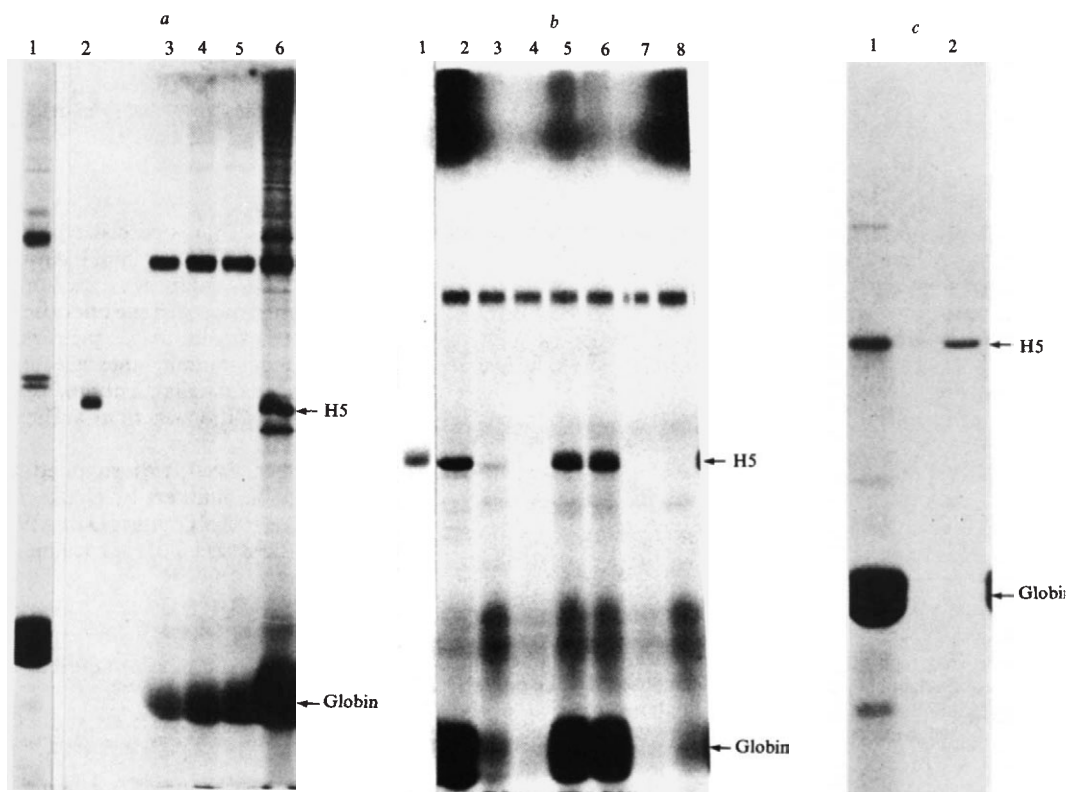
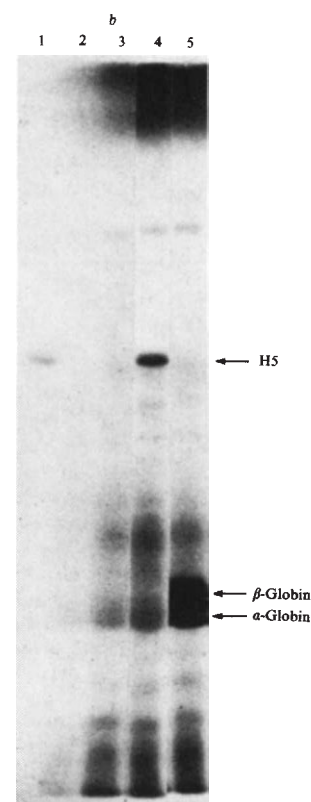
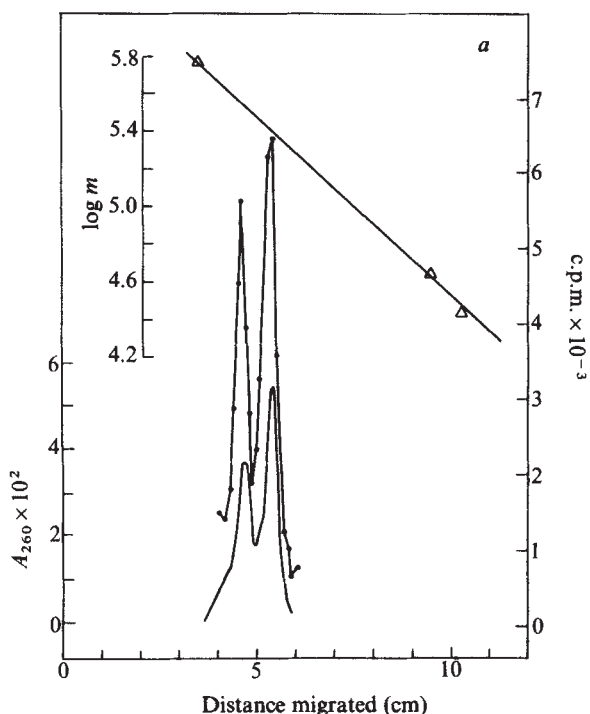


Fig. 2 Preparative electrophoresis of poly(A)⁺RNA. RNA prepared as in Fig. 1b and size-fractionated by two cycles of sucrose gradient centrifugation was separated into poly(A)⁺ and poly(A)⁻ RNA by poly(U)-Sephacrose chromatography. Both fractions were further analysed on 3.5% polyacrylamide, 99% formamide gels²⁷. Labelled 16S and 5S *E. coli* rRNA (extracted from *E. coli* subunits provided by Dr R. Brimacombe) were included as size markers, and calf liver tRNA (Boehringer) as carrier and marker. After electrophoresis, the gels were washed for about 3 h in sterile water and scanned at 260 nm in a Gilford spectrophotometer. The gel was then frozen and sliced into 0.5-mm slices in a Mickle gel slicer. The slices were eluted in pairs in 0.5 ml of 0.6 M lithium acetate, 0.2% SDS²⁸, 5 $\mu\text{g ml}^{-1}$ calf liver tRNA for about



18 h. After phenol extraction and ethanol precipitation, the eluates were dissolved in 10 μl sterile water. Aliquots (1 μl) were used for translation assays as described under Fig. 1a and for poly(U) hybridisation²⁹ with tritiated poly(U) (NEN). *a*, Absorbance at 260 nm (—); poly(U) hybridisation (— · —); 16S, 5S and 4S RNA markers (Δ). *b*, RNA samples separated by electrophoresis as in *a* were analysed by cell-free translation as in Fig. 1a. Lane 1, *in vitro* labelled H5 (ref. 26); lane 2, lysate without RNA added; lanes 3–5, translation products from RNA samples that migrated 4.1, 4.5 and 5.4 cm along the gel shown in *a*. The arrows mark the distances migrated by the respective marker proteins.

product was specifically solubilised by 5% perchloric acid which is known to selectively extract very lysine rich-histones¹⁷; and (3) all the protein-bound ³⁵S-methionine radioactivity extracted with 5% perchloric acid from cell-free assays was immunoprecipitated by purified rabbit anti-H5 antibodies, and co-migrated with H5 on SDS electrophoresis of the immunoprecipitate (Fig. 1c, and manuscript in preparation).

To characterise further the H5 mRNA, total RNA from immunoprecipitated polysomes was fractionated by size by two sucrose gradient cycles and the poly(A)⁺ RNA was separated by poly(U)-Sephacrose chromatography and analysed by electrophoresis in polyacrylamide gels containing 99% formamide. Only two significant peaks were observed when the RNA gel was scanned at 260 nm (Fig. 2a). Translation of fractions eluted from the gel showed a peak of H5 mRNA activity in the more slowly migrating zone (Fig. 2b, lane 4) and mainly β -globin in the faster zone (Fig. 2b, lane 5). No other prominent translation products were observed.

For additional confirmation of polyadenylation of the H5 mRNA, peak fractions of the eluted RNA were hybridised with tritiated poly(U) (Fig. 2a). The more slowly migrating zone showed a degree of hybridisation similar to or greater than that of the globin component. The optical density, the poly(U) hybridisation and the translational activity coincided closely in both peaks. This result (Fig. 2a) leads to an estimated molecular weight of $3.38 (\pm 0.15) \times 10^5$ for the polyadenylated H5 mRNA, that is, a length of about 1,000 nucleotides. A parallel electrophoresis of chicken globin mRNA gave two partly resolved peaks of molecular weights 2.34×10^5 and 2.14×10^5 for the β - and α -chains respectively, in good agreement with the data of Crawford *et al.*¹⁸, but significantly higher than the molecular weights found by Longacre & Rutter¹⁹.

If the H5 mRNA is polyadenylated then it should be possible to isolate and measure the length of the poly(A) since this sequence is relatively insensitive to RNase A and RNase T1.

Aliquots of fractions eluted from near the peaks of both the H5 and globin zones in the preparative gel were digested with RNase A and RNase T1 essentially as described by Brownlee *et al.*²⁰. Parallel RNase digestion in these conditions rendered *Escherichia coli* rRNA soluble in 5% trichloroacetic acid and did not effect the acid solubility of poly(A). The digested samples were electrophoresed in 10% polyacrylamide gels containing 99% formamide, together with 5S and 4S RNA markers. Two parallel gels included oligo(dT) 17 as an additional marker. The gels were scanned, sliced and the RNA eluted and hybridised with labelled poly(U)²⁹. The results (Fig. 3a, b) showed a peak of hybridisation activity for H5 at a mobility corresponding to 150 ± 50 nucleotides and for globin at about 110 ± 40 nucleotides.

The results presented above suggest that a significant proportion of the H5-coding mRNA of chicken red blood cells is polyadenylated.

In the sample analysed, the lengths of the polyadenylate and of the H5 mRNA, together with the protein coding sequence³⁰, lead to the conclusion that in addition to the poly(A) sequence there are about 260 non-translated nucleotides. The relative amounts of poly(A)⁺ and poly(A)⁻ H5 mRNA varied from sample to sample but most of the translatable activity was always found in the polyadenylated material. The binding of H5 mRNA to oligo(dT)-cellulose and poly(U) sephacrose is not an artifact due to the high lysine content of H5 (lysine may be coded by AAA or AAG) because, as is clear from Fig. 3b, a long fragment of poly(A) is present in the H5 mRNA, and, in addition, short stretches of oligo(A) do not contribute to the poly(U)

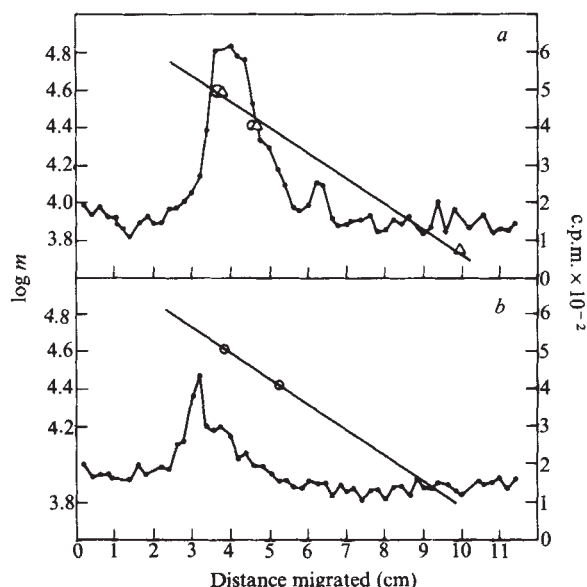


Fig. 3 Poly(A) length measurement. Aliquots of approximately 1 μ l of the RNA eluted from slices at 4.5 and 5.4 cm from the origin of the gel shown in Fig. 2a were digested with RNase A (RNase to substrate ratio 1:100) and RNase T₁ (RNase to substrate ratio 1:10) as described²⁰, in the presence of carrier (0.2 μ g) calf liver tRNA. The digests were made 80% in formamide, mixed with 5S *E. coli* rRNA (from Dr V. Erdman) and calf liver tRNA markers and electrophoresed on 10% polyacrylamide, 99% formamide gels made and electrophoresed as described by Pinder *et al.*²⁷ except that the bis-acrylamide concentration was lowered to 5%. After electrophoresis, the gels were washed, scanned and sliced as described in the legend to Fig. 2. The eluates were analysed by hybridisation with tritiated poly(U)²⁹. *a*, Sample from 5.4 cm (globin); *b*, sample from 4.5 cm (H5); Δ , 5S, 4S RNA, and oligo(dT) 17 markers run on a parallel gel; $\circ$, markers co-electrophoresed. The slope of log *M* versus mobility curve from the two parallel gels was used to draw the lines through the positions to which the markers migrated.

hybridisation. Another possibility is that the H5 mRNA co-migrates with significant amounts of polyadenylated mRNA which translates poorly or not at all under the conditions used. Although we cannot rule this possibility out, we consider it to be very unlikely.

The results presented here conflict with the data of Scott & Wells¹⁶ who isolated H5 mRNA from an oligo(dT)-cellulose run-through fraction. They do not, however, make clear whether any H5 mRNA did bind to oligo(dT) nor did they characterise the putative H5 mRNA activity as we have done. We also find, by *in vivo* labelling and from the yields of the specific immunoprecipitated polysomes, that the H5 mRNA activity does not exceed 0.6% of total messenger activity in anaemic red blood cells (manuscript in preparation), a figure much lower than that estimated by Scott & Wells¹⁶.

The translation of H5 in the absence of DNA synthesis suggests that the H5 gene or the mechanisms that regulate its expression differ intrinsically from those for the other histones. The polyadenylation of H5 may be part of the mechanism allowing this histone's unique expression. The genomic location of the H5 gene and the timing of its transcription are among the questions now being examined.

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***In vitro* simulation of senescence-related membrane damage by ozone-induced lipid peroxidation**

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Recent evidence indicates that as cells deteriorate, increasing proportions of the membrane lipids become crystalline (gel phase) at physiological temperature. Microsomal as well as chloroplast membranes from ageing bean (*Phaseolus vulgaris*) cotyledon and leaf tissue acquire regions of gel-phase lipid as senescence advances¹⁻⁵. Gel-phase lipid has also been detected in microsomal membranes from aged batch cultures of green algae (*Scenedesmus quadricauda*)⁶, microsomal membranes from regressing corpus luteum of rat⁷ and plasma membranes of chick intestinal cells infected with an intracellular coccidial parasite⁸. These observations suggest that the presence of gel-phase lipid in membranes at physiological temperature may be a generalised feature of cell deterioration whether it is attributable to natural ageing or to some kind of infection. Moreover, the resulting mixture of liquid-crystalline-(fluid) and gel-phase lipid in the bilayer is likely to render the membranes leaky^{9,10}, and could thus readily account for the loss of cellular compartmentalisation that accompanies tissue senescence. The present study reports that phase changes occurring naturally during senescence of bean cotyledons^{1,2} can be simulated by exposing isolated membranes from young cotyledon tissue to ozone, a treatment that induces lipid peroxidation¹¹. This finding has considerable bearing on previous observations which implicate atmospheric ozone as a source of crop damage by reason of its ability to induce premature plant senescence¹², and lipid peroxidation as a major cause of membrane deterioration in ageing systems^{13,14}.

Smooth microsomes and cytosol were isolated from cotyledons of 3-day-old etiolated seedlings of *Phaseolus vulgaris* (Kinghorn) grown at 29°C (refs 15, 16). The isolates were exposed either separately or as a mixture to three 2-min pulses of ozone administered at prescribed intervals over a period of 1 h (Fig. 1). Levels of malondialdehyde (MDA) were measured to determine the degree of lipid peroxidation, and phase changes in the membrane lipid were monitored by wide-angle X-ray diffraction. This technique allows unambiguous detection of gel-phase lipid in membranes and can thus be used to determine liquid-crystalline- to gel-phase transitions⁴.

Figure 1a shows ozone concentration fluctuations in the reaction mixture over the 60-min incubation period. These measurements were obtained by bubbling ozone into buffer; corresponding measurements for the full incubation mixture could not be made because the turbidity of the mixture interferes with the spectrophotometric assay for ozone. The buffer was given 2-min pulses of ozone at 0-, 20- and 40-min intervals. Immediately following each pulse the concentration of ozone in the buffer was about 24 p.p.m.; it then decreased over a 20-min period to about 4 p.p.m. (Fig. 1a).

Samples of the incubation mixtures were removed for MDA assays immediately after each pulse of ozone. This 3-carbon dialdehyde is produced during oxidation of polyunsaturated fatty acids^{17,18}, and it is clear that progressively less MDA was produced with each successive exposure to ozone when membrane lipids were the sole source of polyunsaturated fatty acids (Fig. 1b, incubation mixture A). By contrast, the mixture of membrane and cytosol yielded increasing levels of MDA with each ozone treatment (Fig. 1b, incubation mixture B). However, treatment of cytosol alone revealed that the extra MDA

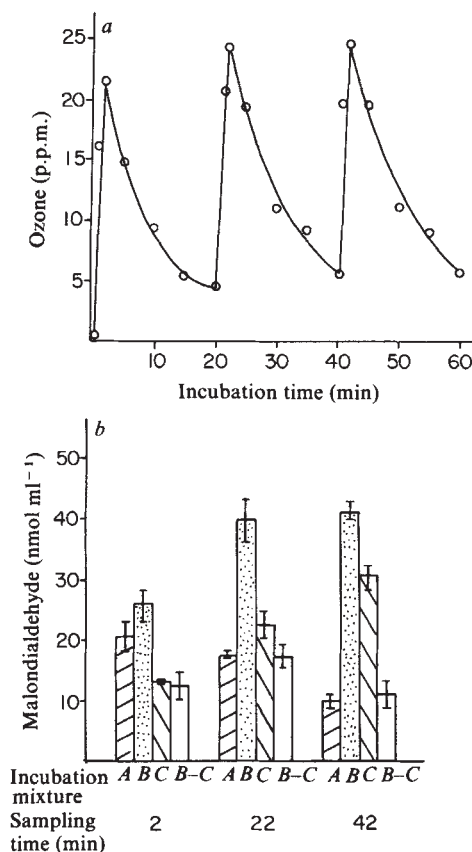


Fig. 1 a, Measurements of ozone delivered to the experimental system. Ozone was generated by passing oxygen at 44 ml min⁻¹ through an electrical discharge ozoniser, and bubbled with a gas dispersion tube through 30 ml of 0.05 M phosphate buffer, pH 7.0, in a 100-ml graduated cylinder placed in a shaking water bath set at 29°C and 130 c.p.m. A 2-min pulse of ozone was delivered at 0, 20 and 40 min of the incubation period, and the total incubation period was 60 min. Samples of buffer (0.2 ml) were taken at intervals shown and assayed for ozone as described³². b, Ozone-induced lipid peroxidation. Ozone was delivered as described above to three reaction mixtures containing: A, smooth microsomes from 3-day-old cotyledons¹⁵ (0.5 mg protein ml⁻¹) and bovine serum albumin (BSA; 2.5 mg ml⁻¹) in 30 ml of 0.05 M phosphate buffer, pH 7.0; B, smooth microsomes from 3-day-old cotyledons¹⁵ (0.5 mg protein ml⁻¹) and cytosol from the same tissue¹⁶ (2.5 mg protein ml⁻¹) in the same buffer; C, cytosol from 3-day-old cotyledons (2.5 mg protein ml⁻¹) and BSA (0.5 mg ml⁻¹) in the same buffer. All the reaction mixtures and a set of identical control reaction mixtures not exposed to ozone were incubated for 60 min at 29°C and agitated at 130 c.p.m. Samples (0.2 ml) of each reaction mixture were taken immediately after each 2-min ozone pulse and assayed for malondialdehyde (MDA) using a modified^{36,37} thiobarbituric acid test³³. The values obtained for the experimental reaction mixtures were corrected for levels of MDA found in the corresponding control reaction mixtures. S.e.m. values are shown; *n* = 3.

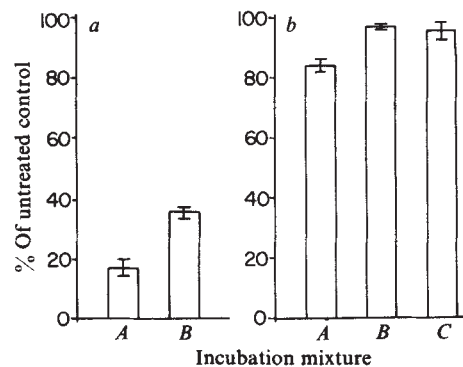


Fig. 2 Levels of phospholipid (a) and protein (b) remaining in the ozone-treated reaction mixtures after 60 min of incubation. Values are expressed as a % of corresponding controls that were not treated with ozone. Reaction mixtures (the same as described in Fig. 1b) were extracted with chloroform/methanol (2:1) and levels of organic phosphate in the extracts were determined³⁴. Protein in the reaction mixtures was measured by the Lowry method.³⁵ S.e.m. values are indicated; *n* = 3-6.

production could be attributed to peroxidation of cytosol lipids (Fig. 1b, incubation mixture C). Indeed, when the cytosol values were subtracted, cumulative levels of MDA produced over the 60-min period indicated that membrane treated in the absence of cytosol sustained more oxidative damage than that treated in its presence (Fig. 1b).

This was confirmed by measurements of phospholipid at the end of the 60-min incubation period. Incubation mixtures containing membrane and cytosol retained twice as much phospholipid as did those containing membrane alone (Fig. 2a). Moreover, lipid seems to sustain much more damage during ozone treatment than protein. For example, the reaction mixture containing membrane in the absence of cytosol retained less than 20% of the original phospholipid after ozone treatment, whereas greater than 80% of the original protein was still present (Fig. 2). The values recorded in Fig. 2b are for total protein and do not reflect the relative fates of membrane protein, cytosol protein and serum albumin on ozone treatment, but the proportions of these three sources of protein were very different in the three types of reaction mixture tested, and in each case there was little decrease in protein resulting from the ozone treatment (Fig. 2b). In addition, the size of the membrane pellet obtained by centrifuging the incubation mixture containing membrane in the absence of cytosol was consistently smaller than that obtained from the membrane-cytosol mixture, and both these membrane pellets were smaller than those obtained from identical control incubation mixtures that were not exposed to ozone. These observations collectively suggest that cytosol partially protects the membrane from ozone damage by sustaining peroxidation itself. Phospholipid and other organophosphorus compounds were not detectable in the cytosol fractions although lipid (presumably triglycerides) could be seen suspended at the tops of the centrifuge tubes in which cytosol was isolated. This is presumably the source of the MDA produced during exposure of cytosol to ozone.

Wide-angle X-ray diffraction of the membrane pellets from control and experimental incubation mixtures revealed that ozone treatment raises the lipid transition temperature (T_c). As might be expected from the results of the chemical assays, the T_c of membrane treated in the absence of cytosol was raised to a higher temperature than that of membrane treated in its presence. The difference between experimental and control transition temperatures was 18°C in the former and only 6°C in the latter (Fig. 3 legend). The diffraction patterns in Fig. 3 demonstrate that the proportion of gel-phase lipid formed is greater in the absence of cytosol than in its presence, and that the amount of gel-phase lipid increases as the ozone treatment is intensified. Indeed, when eight 2-min pulses of ozone rather than three were administered over a 60-min period, virtually all the membrane lipid was in the gel phase at 16°C (Fig. 3e, g).

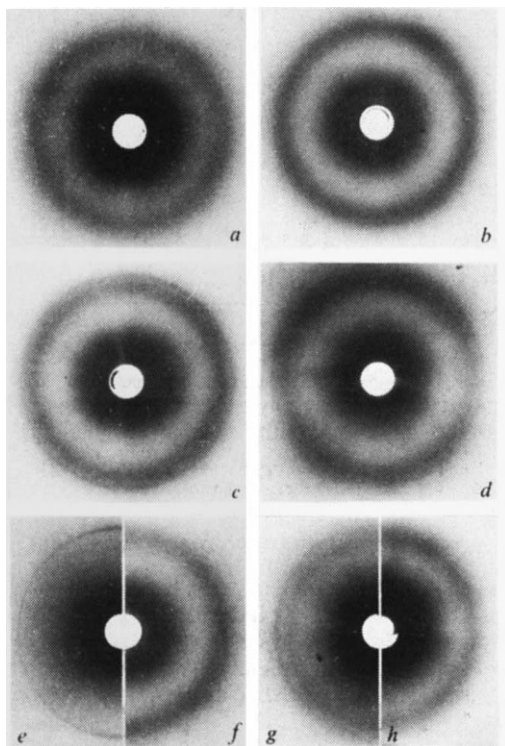


Fig. 3 Wide-angle X-ray diffraction patterns recorded from ozone-treated and untreated smooth microsomes from 3-day-old cotyledons. Reaction mixtures containing membrane and membrane plus cytosol were set up as described for Fig. 1b. Control reaction mixtures were given no ozone; one set of experimental reaction mixtures was given three 2-min pulses of ozone at 0, 20, and 40 min, and another set (in 15 ml rather than 30 ml final volume) was given eight 2-min pulses of ozone at 0-min and at 5-min intervals (up to 35 min) thereafter. After 60 min of incubation at 29 °C the membranes were pelleted from the reaction mixtures by centrifugation at 150,000g for 1 h. Wide-angle diffraction patterns were recorded from the packed membranes as previously described^{1,2}. Patterns recorded at 16 °C for untreated membrane (a) and untreated membrane plus cytosol (b) show (from outside to inside) a diffuse band at a Bragg spacing of 4.6 Å representing liquid-crystalline lipid and a diffuse band at a Bragg spacing of about 10 Å which probably derives from protein but is not well characterised¹. Patterns recorded at 16 °C for membrane (c) and membrane plus cytosol (d) treated with three 2-min pulses of ozone show (from outside to inside) a sharp band at a Bragg spacing of 4.15 Å representing gel phase lipid¹ and a diffuse band at 4.6 Å representing liquid-crystalline lipid¹. Patterns recorded at 16 °C (e) and 55 °C (f) for membrane treated with eight 2-min pulses of ozone show (from outside to inside) a sharp band at 4.15 Å in e and the diffuse liquid-crystalline band at 4.6 Å in f. Patterns recorded at 16 °C (g) and 55 °C (h) for membrane plus cytosol treated with eight 2-min pulses of ozone show (from outside to inside) a sharp band at 4.15 Å and a diffuse band at 4.6 Å in g and only the diffuse band at 4.6 Å in h. Transition temperatures (defined as the point at which gel phase lipid first appears as the temperature is lowered) for the experiments illustrated were: a, 12 ± 1 °C, n = 7; b, 15 ± 1 °C, n = 6; c, 30 ± 2 °C, n = 5; d, 21 ± 1 °C, n = 6; e, f, 52 °C, n = 1; g, h, 47 °C, n = 1.

Moreover, T_c values for membranes receiving this higher ozone dose were 52 °C in the absence of cytosol and 47 °C in its presence. These values for T_c are comparable to those reported previously for the same membranes isolated from extensively senescent 9-day-old cotyledons. Thus, during natural senescence of this tissue, T_c for smooth microsomes rises from 3 °C for 2-day-old cotyledons to 56 °C by day 9 (refs 2, 4, 5).

The specific mechanism by which ozone causes injury to plant tissue is not understood. However, there is considerable evidence that membranes are primary targets. Leaves exposed to ozone display water-soaked spots and become chlorotic and necrotic, symptoms which are thought to reflect loss of membrane selective permeability¹². Exposure to ozone has been shown to cause leakiness in leaf cells¹⁹, potato tubers²⁰, unicellular algae²¹ and isolated organelles^{22,23}. Several observations support the proposal that ozone damage accrues in part from oxidation of unsaturated fatty acids in membranes. Malondialdehyde accumulates²⁴⁻²⁶, and there are small

decreases in the levels of polyunsaturated fatty acids following exposure of plant tissue to ozone²⁶. Lipid peroxidation also renders liposomes leaky^{27,28} and increases their viscosity²⁹. Our observation that exposure to ozone induces the formation of gel-phase lipid offers an explanation for this increased leakiness, for it has been previously demonstrated that the co-existence of liquid-crystalline- and gel-phase lipid greatly increases the permeability of bilayers^{9,10}. The finding that the formation of crystalline lipid during senescence can be simulated by treating membranes from young tissue with ozone supports the contention that lipid peroxidation plays an integral role in senescence, and complements previous observations that anti-senescence compounds such as cytokinins confer resistance to ozone damage on leaves³⁰ and lower the endogenous level of lipoxygenase³¹.

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Erratum

In the letter 'Do sponges help hold coral reefs together?' by J. L. Wulff and L. W. Buss, *Nature* **281**, 474-475, in the footnote to Table 1, in line 6 'does assist with the' should read 'does not provide any'. The last two sentences of the penultimate paragraph should read 'Although the influence of bioerosion should not be underestimated, neither should the binding capacities of other reef sponges. Demosponges clearly require several terms in the reef carbonate balance equation.'

BOOK REVIEWS

Biological oceanography

J.H.S. Blaxter

SEVENTY per cent of the Earth's surface is covered by sea; the *average* depth of the ocean is 3800 metres; its maximum depth is 11,500 metres; its volume is 130 million km³. These statistics give some idea of size of the last relatively unexplored regions of the Earth, sometimes called "inner space" by the popular press. The science of oceanography (or perhaps oceanology is a better term) started in earnest only 100 years ago during the first Challenger Expedition from 1872–76.

Professor Marshall's book reviews the current level of knowledge of biological oceanography. He attempted in the first instance to revise his 1954 book *Aspects of Deep Sea Biology*. So much had occurred, however, in the past 25 years he set aside his "paste and scissors" (and what a crime it would have been to dissect that volume), and started afresh. The first action of the reviewer was to take the 1954 book from his shelves and compare it with the new one. The latter has 50% more pages which probably indicates an increase of 50% in the volume of knowledge over 25 years. Some of the figures, drawn by Olga Marshall, are fortunately repeated but many are new.

The sections cover Deep Sea Environments; Sea Life, including oceanic plants, pelagic animals and life on the deep sea floor; Deep Sea Food Webs, including those in the pelagic zone, near the deep-sea floor and of the deep sea floor; Physiological Aspects, with chapters on buoyancy, bioluminescence, sensory systems and early life histories; and Biogeography. There is a good bibliography and extensive Species and Subject indexes. There are 170 excellent figures drawn by Olga Marshall and others, and two colour plates by Olga Marshall, the originals of which were hung in the Royal Academy Summer Exhibition.

The book conveys well the esoteric world of the deep sea. The uninitiated will be amazed at the variety and adaptations of oceanic organisms. The initiated will always remember the experience of seeing their first deep sea haul, not only the bizarre forms, but the variety of species and the paucity of the biomass — half a bucket is usually enough to contain the catch from four hours fishing. For every 10 metres depth the pressure increases by one atmosphere; hence many of the animals are living at pressures of hundreds

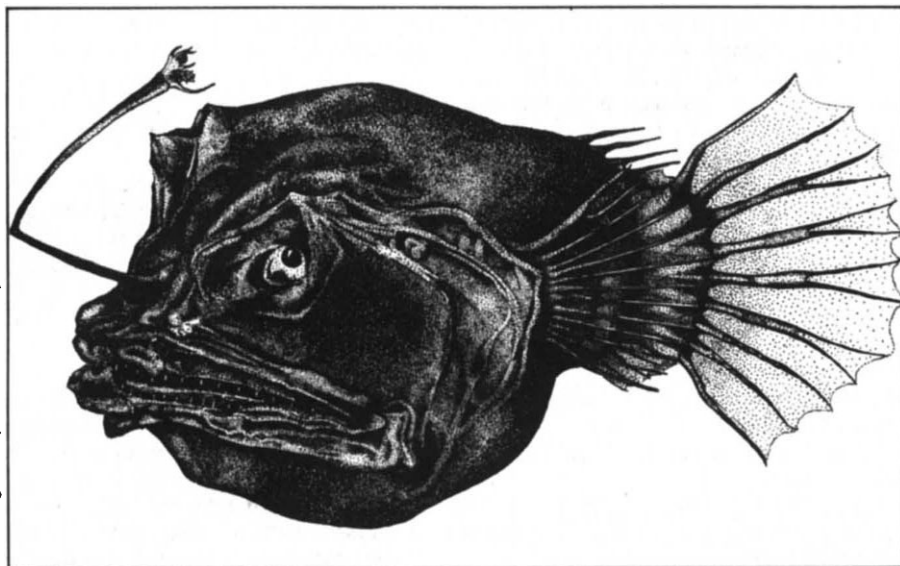
Developments in Deep-Sea Biology. By N.B. Marshall. Pp. 566. (Blandford: Poole, UK, 1979.) £15.

of atmospheres or thousands of pounds to the square inch. Below 1000 metres it is dark except for flashes of bioluminescent light produced by the animals themselves; even above 1000 metres the light is dim and a monochromatic blue. Below the thermocline, which is usually sharp and acts as a barrier both to animal migration and the exchange of water and nutrients, the temperature is an unfluctuating 1–4°C with no seasonal changes. It is the lack of daily and seasonal changes which make the environment so homogeneous. The possibility of lateral distribution without severe physical, chemical or topographical barriers is the reason why deep sea hauls look so similar, whatever part of the ocean is being fished. The ocean bed, however, is not uniform. Submarine mountain ranges run up the centre of the main oceans; these are the areas where tectonic plates originate which are involved in the separation of the continents. Between the continental shelf and the mid-ocean ridges lie ocean basins, many covered with deep sediments forming abyssal plains.

Professor Marshall describes biological life in the pelagic zone and in the depths. This section of the book is much longer than the previous book, reflecting the

advances made since the War. This is essentially a review and in no sense a key to identification. The adaptations can be clearly linked to the shortage of food (large rapacious mouths, recurved teeth, highly distensible stomachs) and the need to attract prey or mate (bioluminescent lures and photophores). The downwardly pointing photophores of some deep sea fish are almost certainly a means for breaking up their form to predators looking up and searching for food in silhouette against the dim downwelling light.

The three chapters on food webs lead to the description of vertical migration, found to be a surprisingly regular and worldwide phenomenon wherever investigations have been made. This rise at dusk to the surface and sinking at dawn towards the sea bed are found in very divergent animal groups and seem to be linked to feeding and the avoidance of competition and predators. Non-migrant related species often occupy separate depth strata, whereas vertical migrants, being able to exploit different depths, often have coincident depth ranges. Although much was known about faunistic variation and the taxonomic make-up of deep sea organisms from earlier investigations, the past 25 years (while bringing some new species to light) has been marked by a better understanding of depth distribution. This is more significant than might be



Female angler-fish (*Oneirodes eschrichtii*)

N.B. Marshall, redrawn from Pietsch, 1974

apparent. It has required great ingenuity to develop equipment to fish at specified depth without "contamination" on the way down and on the way up, before and after the haul. It was disappointing to find that Professor Marshall had not included a section on fishing gear (though he quotes a recent reference) because great advances have been made in this field.

Studies on physiology have also been advanced significantly, although deep sea organisms, especially fish, are difficult to catch in good condition. The physiological discoveries have mainly resulted from a study of functional anatomy and biochemistry, both of which are possible on moribund or even newly-dead animals or on particularly hardy species. A whole range of buoyancy adaptations have been discovered such as low density (for example, ammonical) body fluids, high lipid flotation, reduced skeletons, and special pneumatic float and swimbladder modifications. The sensory systems may be specialised with upwardly directed "telescopic" eyes of high sensitivity with a banked retina of pure rod visual cells. The chemosenses and lateral line may also be elaborated. Life histories have to be maintained on impoverished resources. Often deep sea organisms have no breeding season and produce large eggs which yield larger more viable larvae, and these often rise to the surface during a distributional phase.

Professor Marshall unfortunately says little about oceanic resources, describing the deep ocean as a deserted living space in comparison with the highly productive areas of the continental shelf. Nevertheless, it is true that some of the new species likely to be exploited are further offshore. Apart from lantern fish, which are truly a deep water potential resource, squid, krill and blue whiting are all offshore species found over deep water.

The advances made in biological oceanography since the War must have been expensive: an ocean-going research vessel now costs several thousand pounds to run each day. In the current climate of recession, research vessels may be laid up and it is probable that the next 25 years may not be so productive as the last. Professor Marshall's book has probably been produced at a key time at the end of an almost logarithmic increase in research effort. He is one of the most informed of our deep sea biologists and his book is an important addition to the literature of biological oceanography. It has his characteristic style and it should find a place in the libraries of Universities, Institutes and individuals, as it has the merits of timeliness and readability as well as a modest price. □

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Ancient climates

W.B. Harland

Climates Throughout Geological Time. By L.A. Frakes. Pp. 304. (Elsevier Scientific: Amsterdam and New York, 1979.) \$58.50; Dfl. 120.

SEVERAL books have been published recently on the theme of ancient climates and especially of ice ages. Each has distinctive shortcomings and in every case there is the human limitation that prevents any one person from mastering more than a part of the vast relevant literature. Taking this into account Frakes has without doubt written the best. It is a serious study without journalese or padding and it reflects the author's own work over a wide range of the subject matter. It is a thorough attempt to integrate the latest palaeogeographical reconstructions and, results from the Deep Sea Drilling Program with ^{18}O investigations on palaeotemperatures, by an author with long experience investigating tillites of various ages in more than one continent. Throughout Frakes has carefully considered what is known about the likely properties and motions of the air and water masses enveloping the Earth in relation to the changing pattern of its land and sea. The whole work is internally consistent, having gone through one mind. (In this field too many authors have attempted to support their own prejudices with compilations of undigested data.) A point of view emerges that recurs and has some of the following characteristics.

Frakes gives only 10 out of 267 pages of text to Pleistocene events and he mistakenly includes Pleistocene as a division (the last 1.6 to 2.0 Myr) of the Neogene Period. Such a slip in writing down the Quaternary glaciations would be unthinkable to those brought up in the Northern Hemisphere where earlier ice ages are often treated as an appendix to The Great Ice Age. So this work usefully counter-balances our Arctic preoccupations and brings the southern oceans into play as they developed around Antarctica. There during the last 30 Myr is evidence of ice and a major ice sheet for at least half that time, ice sheets in the northern hemisphere developing only around 3 Myr ago. Although Frakes shows himself to be uncharacteristically ignorant of Pleistocene stratigraphy (and so not able to assess critically the Milankovitch cycles) the reviewer welcomes this refreshing approach that interprets the present as a transient phase evolving out of the past.

Throughout the work there is a continual, at times almost irritating, equivocation over interpretation as the author meticulously discusses each successive problem. So little can be concluded with certainty in considering ancient climates because of the complexity

of interacting systems each with negative and positive feedbacks. For example, the interplay of solar constant, ocean gyres, thermal gradients, polar fronts, global albedo, atmospheric CO_2 , carbonate compensation depth, biological activity and distribution, ^{18}O , evaporation and precipitation as ice or water, eustasy and tectonics provide the characters in an everchanging choice of scenarios for each episode of history. The first chapter sets out the relevant parameters from atmospheric and oceanic circulation but the applications are repeatedly discussed in context throughout. This explains how it is that many of the questions of palaeoclimatology that were asked more than 100 years ago remain enigmatic.

On the other hand equivocation over tillites and other evidences of ice has virtually been eliminated. The story of ice ages is the skeleton of climatic history and for this Frakes is as well equipped as any from his own researches. There is little new in the account of the broad pattern of glacial history but its integration into the stratigraphical record is thorough. No glacial deposits are recorded older than about 2700 Myr and the reason for supposing the Earth to have been warmer during the preceding 2000 Myr are familiar but well marshalled. A run of well described glacial events between about 2700 and 2100 Myr lack critical age or palaeogeographical control.

Another cluster of very widespread late Precambrian tillites occupies the time span between about 900 and 600 Myr. The reviewer especially welcomes Frakes' clear conclusion that the evidence for part of this time, if for no other, requires there to have been low latitude glaciation. He now rules out the prevailing view to which he was earlier sympathetic that the wide distribution of these deposits could be accounted for by the imprint of a wandering pole. He then has to choose between either a global refrigeration that would be difficult to explain without a reduction in the solar constant, or a radically different obliquity at that time (for example, 54° instead of 23.5°). This could be ruled out if high latitude glacial deposits were to be established, for the essence of the obliquity explanation is that with Earth's poles pointing nearer to the Sun only low latitudes could be glaciated.

Phanerozoic history that follows is probably accountable in terms of terrestrial parameters. Palaeozoic ice sheets relate to the southern continent Gondwanaland containing the South Pole and this is a field in which Frakes is a long-standing authority. There may have been ice (possibly continuous) there throughout most of Phanerozoic time (600 to 240 Myr) but the well established ice ages occurred at the end of Ordovician time (435 Myr) and through much of Carboniferous and Permian time (say 330–240 Myr). The highest temperatures have been Devonian (400–350 Myr). The lack of ice in the

Mesozoic Era is fully discussed in terms of oceanic distribution and low albedo leading to peak temperatures in mid-Cretaceous time (say 100 Myr), since when a fluctuating decline ensued.

Frakes' work is scholarly; not every point of opinion is referenced; this may irritate some, but too frequent a punctuation with brackets would have been more irritating. There is, however, an adequate supply of references in the text to most data to be used, with a list at the end of about

500 to 600 works and an adequate index. This book establishes palaeoclimatology as an inseparable part of stratigraphy, itself the documentary basis for understanding most aspects of Earth history. □

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Primordial germ cells

C.C. Wylie

Primordial Germ Cells in the Chordates. By P.D. Nieuwkoop and L.A. Sutasurya. Pp.187. (Cambridge University Press: Cambridge, 1979.) £14.50.

THE establishment of the gonad in early vertebrate embryos has fascinated developmental biologists and cell biologists for well over a century now. Basically, the problems fall into the following categories: (1) how is the germ line determined in the early embryo?; (2) what is the mechanism by which the primordial germ cells (PGCs) migrate from the site of their determination to the site of gonad formation?; and (3) what is the mechanism by which PGCs are guided to their target? Thus, many of the cellular processes which make development such a fascinating process are encapsulated in this

particular piece of organogenesis, and this has been reflected in the considerable research interest in this subject.

It is therefore timely that Professor P.D. Nieuwkoop's book about the PGCs of the chordates has appeared, for this is a thorough and clear survey of work in this field over the past hundred years. However, despite the authors' considerable contribution to the field, and some five hundred cited pieces of work, we are still only able to answer the above three fundamental questions in the most general way.

Professor Nieuwkoop's main contribution, and indeed, the main pre-occupation in his book, is the site and manner of determination of the first germ line cells (the "presumptive" PGCs). The fact that these seem to differ in two vertebrate groups which have been closely studied, the anuran and urodele amphibians, leads him to speculate that the evolutionary tree of the vertebrates, particularly with respect to the amphibians, may be more complex than has been hitherto suspected.

The study of taxonomy through careful

study of ontogeny has a long and honourable history, and has tended to be neglected of late. This is largely due to a focussing of research interest on to the cellular and molecular events of embryogenesis, in an attempt to define causative factors in cell differentiation. It is therefore a timely reminder by the author that molecular and cellular phenomena of development are just as important clues in taxonomy as are anatomical features.

This concentration on the taxonomic aspects of the PGCs in chordates is both a strength and a weakness. The weakness is simply because the data on the establishment of the germ line cells, and the route they take to the developing gonad, are still only documented in a fragmentary and unsystematic way. The middle section of the book contains a truly excellent description of this. However, when examined critically, hard evidence on the means by which the germ line is established in chordates only exists for a small number of amphibian species, and not at all for the amniotes, making this a rather tenuous reason for tinkering with the chordate evolutionary tree. The strength of this approach, however, is that it is bound to stimulate research in this subject, particularly into those groups which so far have been neglected. This, in turn, will fulfil Professor Nieuwkoop's aim to gain insight into the way in which the causative factors in early development have themselves changed during vertebrate evolution. □

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Plasma proteins

M.J. Geisow

Plasma Proteins. Edited by B. Blomback and L. Hanson. Pp. 401. (Wiley: Chichester, UK, and New York, 1979.) £22.50.

SWEDISH readers will already know about this book because it first appeared in Swedish in 1976. The latest edition of this multi-author volume is a revised version in English and now includes some literature references up to 1978. The promoters are KABI AB, the Swedish firm which supplies plasma protein fractions.

Plasma Proteins is described as an educational text and a clinical handbook. This seems to be a fair description. The introductory chapter by Kai Pedersen is a small nugget of biochemical history. Apart from exposing the roots of plasma protein science, it also deservedly, but not selfishly, establishes the Swedish contribution to the field. The remaining chapters form a comprehensive account of the

biochemistry, pathology and methodology of plasma proteins. The treatment of this material is similar to that in the more expansive work *The Plasma Proteins* (F.W. Putnam, ed., Academic: New York; 1975). In contrast with the latter work, however, you might consider reading the KABI book from cover to cover.

Inevitably for a textbook produced several years ago, there are omissions. For instance, the recent considerable advances in the three-dimensional structure of lipoprotein have not been incorporated in the otherwise excellent description. Nevertheless, as an educational text, the chapters are informative and easy to read. Despite multi-authorship, the closely-linked subjects of defence and repair (that is, the antibody/lymphocyte and blood clotting systems) are treated in a unified fashion.

At a molecular level, there is a pleasing emphasis in this book on the structures of plasma proteins. For example, there are good descriptions of the functions of the transport proteins albumin and haptoglobin in relation to what is known

about their molecular structures. A similar molecular structure/biological function synthesis is achieved in the chapters on the protective proteins fibrinogen and plasminogen.

The bench worker is catered for by the thoughtful inclusion of much useful physical and chemical data relating to the individual proteins described. Both historical and current purification techniques are given and there is a discussion of industrial methods for the fractionation of plasma.

At a more clinical level, hospital biochemists have been given a generous amount of space in which normal and pathological states of plasma protein chemistry is covered. There are short but clear descriptions of the main diagnostic plasma protein analytical techniques, with electrophoretic separations in full colour.

I expect to see this book above the benches in the teaching hospitals — both in the pathology labs and in the medical schools. □

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Invertebrate vision

G. A. Horridge

Handbook of Sensory Physiology. Vol. VII/6: *Comparative Physiology and Evolution of Vision in Invertebrates. A: Invertebrate Photoreceptors*. Edited by H. Autrum. Pp. 728. (Springer: Berlin, Heidelberg and New York, 1979.) DM320; \$176.

THIS splendid and authoritative series has so far been mainly on vertebrates except where invertebrates illustrate primary processes such as transduction, which was extensively reviewed in Vol. VII, Parts 1 and 2. Comparative studies of photoreceptors by authors who work on insects fill Part 6A but still hardly touch the diversity on invertebrate vision. Final coverage cannot be reviewed because two more sections of Vol. VII, Part 6 are expected.

Despite the emphasis on insects there is a wide spread. The refreshing article by Diehn on photic responses of Protozoa accentuates a need for a large protozoan that can be analysed as a single animal using numerous techniques. The negligible fraction of visual pigment and the small size of *Euglena* really limits advance. The thoughtful reviews by Miller of optical filters classified by mechanism and by Snyder on light gathering mechanisms are models of the comparative approach at its best. We almost see the thought processes of the Creator as an optical engineer. Hamdorf's masterly account of visual pigments also

gives the impression that so much is now known that we can imagine that most of the diversity is catalogued and the basic physics is understood. For that reason new students easily get the impression from the volume as a whole that the topic is cleaned out, but of course that is not so. For example, the action of any thick lens system such as an eye is understood in terms of principal planes and nodal points, but judging by this volume these are not known for any compound eye: in many eyes even the location of the lens is not known.

The discrepancy between the detailed, almost final, accounts of arthropod visual cells and our relative ignorance about other photoreceptors is emphasised by two splendid reviews: one by Bennett on the entrainment of diurnal rhythms with the conclusion that the receptor is usually not the eyes; and the other, by Yoshida, on receptors for light outside the eyes. It is difficult to understand why one animal group seems to extract the maximum information from its visual system while another seems to rely on a totally ineffective system like a dermal light sense. Why are they not all obviously at the optimum?

The book is, of course, full of in-jokes for the initiated; Stavenga's article doubly so, perhaps from the Dutch. I particularly like his caution that Swihart's "way of reflecting on butterfly eyes must be treated with care" (page 401). Snyder especially recommends an article (1969) by Kirschfeld but omits the reference. The unique index enables one to see at a glance that only two substantial contributors, Steve Shaw and Sigmund Exner, never published with co-workers, and that of

the works of Exner, only his book is read today.

The 'classical' works of the past are praised in this volume but sometimes we find a misplaced trust in them and sometimes a neglect of their value. An example of the latter is the "thorough investigation of the butterfly visual sense cells" quoted by Stavenga. By measuring the reflection Bernard "made the startling discovery of a red absorbing visual pigment of which the spectrum peaks at about 610 nm". If he had read Max Schultze's classical paper on the compound eye written in 1868 Bernard would have related his red reflections at least partly to the strings of red pigment in the retinula cells. We cannot, however, always believe the classical authors. For example, Exner interpreted these red strings as additional out-of-focus rhabdoms. This was all noted by Ribi in a paper which Stavenga actually quotes in his references but does not incorporate. In fact, in every corner of the subject, as in all science, there are not only great gaps in our knowledge awaiting future genius, there are plenty of murky corners to be cleared out, also requiring lucidity and experiment. One notes the pietism on page 417: "One may assume from the present high interest in photoreceptor physiology and the accordingly rapid accumulation of new data, that some crucial questions will soon be answered"; but interest and data are not the whole story. □

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Silica chemistry

D. Dollimore

The Chemistry of Silica: Solubility, Polymerisation, Colloid and Surface Properties, and Biochemistry. By Ralph K. Iler. Pp.866. (Wiley: New York and Chichester, UK, 1979.) £39.50.

RALPH ILER is well known for his research publications and for his earlier book. *The Colloid Chemistry of Silica and Silicates* (Cornell University Press: Ithaca, New York, 1955). This new book will be judged by a comparison with the old. It must be stated that the author first intended this new venture to be an updated second edition of the earlier book. It must, however, in this expanded form be regarded as a new work.

The purpose of the present book is to provide an account of the chemistry of silica, including soluble silica and silicate

precursors of soluble silica, the polymerisation to polysilicic acids, colloidal sols and gels, and the surface chemistry of silica. This provides the reason for the provision of the subtitles shown above. The consideration of some 2,500 new references since the first book make the comment of the author that science students graduate with little knowledge of silica's properties or chemistry very pertinent. It may be that this book will be an incentive for the provision of more silica chemistry in the undergraduate syllabus. The author states that the book is written for those engaged in work on silica, as a guide to the literature and for those in other fields who need specific information on the subject. The author does this in a book of 866 pages.

In a short introduction Dr Iler describes previous books and reviews of silica chemistry. He also defines terms such as Silanol, polymerisation and silica. Other names are, however, explained at the point of introduction in the text.

The subject is introduced by the

consideration of the solubility of different forms of silica, the factors that influence dissolution and deposition, and the other aspects of the solution chemistry of silica. The various analytical methods are described and compared, and recommended methods given in detail.

Water-soluble silicates are then described as a prelude to the chapter on the polymerisation of silicic acid. A silica sol formation mechanism is clearly detailed together with the necessary factors that control the properties of the system. The various types of gels and powders are characterised, with a great deal of attention paid to their adsorption properties and their uses. There is a chapter on the role of silica in biology, in which the aspects of the occurrence of silica (including toxicity) in mammals and other life forms are discussed. □

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